Pollination ecology of Tasmanian leatherwood (*Eucryphia lucida* Eucryphiaceae Labill.) and the impacts of hive honeybees

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Certificate of Originality

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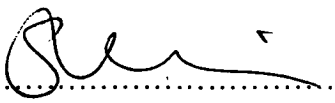
(signed)  (27 February 2001)

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Abstract

Tasmanian leatherwood (*Eucryphia lucida* Labill.) is a tall (up to 30 m) native tree occurring as a canopy co-dominant in Tasmania's cool temperate rainforest. A large proportion (*ca.* 37%) of *E. lucida's* total distribution occurs within the Tasmanian Wilderness World Heritage Area. The nectar of *E. lucida* is used in the production of leatherwood honey and is highly sought after by commercial apiarists.

I investigated the impacts of commercially managed honeybees on *E. lucida* and its native pollinators. Because the type and severity of honeybee impacts are intimately related to the pollination system of the forage species, I also examined aspects of the pollination ecology of *E. lucida*.

Flowers of *E. lucida* are relatively long-lived (12-13 days) and protandrous, with around 6-7 days of pollen presentation followed by 6 days of stigma receptivity. However, the degree of overlap between the male and female phases of anthesis depends on the rate at which pollen is removed from anthers by insect visitors (i.e. flowers are facultatively protandrous). *E.lucida* flowers secrete a relatively dilute nectar (*ca.* 20% sugar wt/wt) from nectaries at the bases of the stamens. Nectar is secreted continuously, although secretion rates are substantially lower at night. Flowers typically contain small volumes of liquid nectar in the early morning which on warm days is rapidly concentrated through evaporative water loss to > 60% wt/wt. This concentrated nectar is highly attractive to insects and flowers typically receive multiple insect visits over a single day.

E. lucida is partially self fertile. Fruit and seed set in bagged flowers which received a superabundance of autogamous self pollen (34% fruit set and 16 % seed set) was relatively low compared to fruit and seed set in un-bagged flowers (80% fruit set and 36% seed set). Stigmas of un-bagged flowers carried large amounts of pollen (estimated at 1700 grains/stigma), and *E. lucida* flowers do not appear to be pollen limited.

Flowers of *E. lucida* received visits from a broad range of native diurnal insects (dipterans;16 families, coleopterans; 6 families, hymenopterans; 5 families, and lepidopterans; 2 families) and nocturnal insects (tipulid flies, elaterid beetles, blattellid cockroaches, and geometrid and pyralid moths), as well as from the introduced honeybee. *E. lucida* flowers also supported a range of squatter insects which used the flowers as a semi-permanent refuge (mainly thrips, staphylinid beetles, and spiders). Visitation rates varied enormously between

sites, ranging from < 2 to > 25 visits per flower per 10-hour day. Nocturnal visitation rates were < 2 visits per flower per 10-hour night. Large dipterans and large coleopterans appeared to be the most important native pollinators of *E. lucida*.

E. lucida appears to be well adapted for maximising pollination under conditions of temporal and spatial heterogeneity in the native pollinator service. Nectar production is independent of temperature, humidity and local shading, and flowers rapidly accumulate nectar sugar on cold days when insects are inactive. *E. lucida* flowers do not reabsorb accumulated nectar sugar. In contrast, the rate of anther dehiscence is strongly and positively dependent on temperature above 10°C, so that pollen release is retarded on cold days. The resulting patterns of nectar and pollen release appear to maximise both male and female function in *E. lucida* flowers under a broad array of weather and pollinator-abundance conditions.

I examined the impacts of hive bees at 13 sites, 7 in the vicinity of a commercial apiary and 6 control sites located >2 km from the nearest apiary (hive bees foraged within 2 km of hives during *E. lucida* flowering). Honeybee activity at flowers was significantly higher near apiary sites compared to control sites, although the mean increase (by a factor of 2.5) was relatively modest. This increase in honeybees resulted in a significant depression in the availability of nectar sugar in flowers around apiaries. Hive honeybees appeared to be excluding feral honeybees from the vicinity of apiary sites. However, there was little evidence that hive bees caused a decline in the visitation rate or abundance of native insects, apparently due to very low numbers of native insects and a superabundance of nectar sugar at some of the sites. However, hive bees may reduce the number of native insects visiting *E. lucida* flowers at a subset of rainforest sites with abundant native insects and low levels of available nectar sugar.

E. lucida flowers were also depleted of pollen more quickly at apiary sites compared to control sites, resulting in a 17% reduction in the standing crop of pollen in male flowers in the vicinity of apiaries. Fruit set tended to be higher near apiaries, although there was no difference in the number of pollen grains on stigmas, fruit dehiscence, fruit weight or seed set between apiary and control sites. Therefore, despite removing pollen more rapidly and reducing the availability of pollen in male flowers, hive bees appeared to have little net impact on the reproductive performance of *E. lucida* trees.

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General Introduction

Impacts of managed and feral honeybees

The European honeybee (*Apis mellifera* L.) is an exotic species in Australia and, as such, may pose a significant threat to native species and natural systems that have evolved in its absence (Pyke 1990). The question as to whether or not honeybees have a detrimental effect on Australia's native biota has been posed for many years, and in recent times has led to substantial though not always constructive debate (Manning 1997; New 1997). Recent research in Australia as well as overseas has shed some light on the nature and extent of honeybee impacts (see the review by Paton 1996). However, as the number of studies has increased, it has become increasingly evident that the potential for and significance of any impacts is contingent on a complex array of factors. This complexity makes the question of whether or not honeybees are detrimental to native biota extremely resistant to generalisations. Indeed, attempts to generalise and impose blanket prescriptions, rather than simplifying the issue, frequently have the unfortunate consequence of polarising arguments into the meaningless extremes of 'for' or 'against' honeybees.

The question of honeybee impacts is complicated by the fact that honeybees foraging in native vegetation may be either feral (i.e. from wild colonies which have established themselves in the bush) or managed bees from commercial or hobby hives (New 1997). Feral colonies occur in most non-alpine vegetation types in Australia where there is available water (Paton 1996). Unlike managed hives, feral honeybees remain in an area year-round during conditions of both nectar abundance and nectar dearth. As a result, feral colonies tend to be relatively small (< 10 000 bees per colony) and less robust than managed hives (Paton 1996). If one assumes that feral colonies have reached some form of equilibrium with their environment, then many impacts of the initial invasion into native vegetation may have already occurred and be no longer detectable. The issue of feral honeybees and their potential impacts has received very little attention, due to their ubiquity in the bush, the relative inconspicuousness of the actual colonies, and to the difficulty in scientifically demonstrating significant impacts (New 1997).

In contrast to feral bees, managed bees are typically moved in and out of an area on a short term basis in order to utilise the intermittent flowering of forage species (Manning 1997). This nomadic aspect of commercial apiculture has a number of important implications for (managed) honeybee impacts and attempts to study them. In contrast to feral honeybees, managed hives are not likely to be in any form of equilibrium within the native vegetation to which they are introduced. Managed hives are typically maintained in a very healthy state by

the apiarist so as to maximise the hives' foraging capacity when they are moved into place during honeyflow. Managed hives may contain as many as 50-60 000 foragers per hive (Paton 1996), while the number of hives involved in an individual apiary is often large (50-150 hives), so that an individual apiary may involve upwards of 75 million foraging bees. Furthermore, hives are typically removed once flowering falls away from its peak (Manning 1997), so that managed hives do not suffer the periods of nectar shortage to which feral colonies are subject (Paton 1996).

Clearly therefore, the introduction of commercial loads of honeybees into an area represents a sudden, massive injection of a foreign species into an area in numbers which far exceed those (i.e. feral honeybees) which can maintain themselves year-round. This in turn suggests that hive bees may be more likely than feral bees to impact on natural systems. However, acting against this assumption is the possibility (frequently cited by apiarists) that nectar supplies during conditions of honeyflow are in excess of that required by the native fauna, with honeybees effectively removing only the surplus nectar (Manning 1997; New 1997). Under this argument, honeybee impacts may be most apparent during conditions of nectar shortage, which would clearly be a feature more of feral rather than of managed bees. Similarly, unlike hive bees, feral honeybees occupy naturally occurring tree hollows which might otherwise be utilised by native birds or mammals (Pyke 1990; Oldroyd *et al.* 1994). Potential competition between honeybees and native species for nesting hollows therefore applies only to feral colonies of bees, although hives may provide a source of feral colonies through swarming.

A further important distinction between feral and managed honeybees is that feral honeybees are present throughout suitable areas of native vegetation, while commercial hives are introduced only to those areas which are accessible to the vehicles used to transport the hives. Thus any putative impacts of commercial hives are likely to be more limited in extent than those of feral colonies. The fact that only accessible areas are utilised for commercial apiculture also has important implications for investigating impacts of managed as opposed to feral honeybees, as large tracts of vegetation closely resembling the utilised areas remain free of hive bees. These inaccessible areas can be used as controls in the experimental design. The latter point is particularly relevant to Tasmania and the leatherwood honey industry as very large areas of *E. lucida*-rich forest are inaccessible to commercial apiculture operations but can be utilised as control sites for comparison with sites in the vicinity of commercial apiaries.

Previous studies on honeybee impacts on native fauna

The study by Pyke and Balzer (1985) was the first manipulative study to examine impacts of honeybees on native bees in Australia, and has been largely responsible for the recent upsurge in interest in the impacts of honeybees on Australia's native biota. Pyke and Balzer (1985) interpreted their findings to suggest that competition occurred between honeybees and native bees, with the numbers of native bees apparently declining in the presence of honeybees at flowers. However, limitations in the experimental design of their work somewhat weakened the strength of their claims for honeybee impacts (Paton 1996). A subsequent study by Sugden and Pyke (1991) examined the impacts of hive bees on the reproductive performance of a native bee, *Exoneura asimillima*, and concluded that hive bees had a negative effect on local colonies of *E. asimillima* as a result of competition for nectar. However, Sugden and Pyke (1991) did not monitor nectar levels at their experimental and control sites, and their study was also limited by a lack of replication in experimental and control plots. A more recent study by Schwarz and co-workers on the impacts of honeybees on the native bee *E. bicolor* (Schwarz *et al.* 1992, Schwarz and Hurst 1997) employed a more robust experimental design including good site replication and the experimental mimicking of both commercial and feral levels of honeybees. Preliminary results of the study indicate that neither commercial nor 'feral' levels of honeybees had a significant negative impact on *E. bicolor*. In fact, colony survival and brood production tended to be higher at sites with hives compared to sites without, possibly due to prey satiation of insect predators in the vicinity of hive bees (Schwarz *et al.* 1992; Schwarz and Hurst 1997). Unfortunately, the levels of nectar and pollen at the study sites of Schwarz *et al.* were not monitored, so it is not known whether the introduction of hives actually led to a significant reduction in floral resources available to *E. bicolor* (*cf.* Paton 1996).

The most comprehensive studies to date on honeybee impacts on Australia's native fauna were carried out by David Paton in two conservation reserves in South Australia. Paton (1993, 1996) examined the impacts of hive honeybees on the foraging behaviour and territory size of the principal pollinator of *Callistemon rugulosus*, New Holland honeyeaters (*Philidonyris novaehollandiae*) at Scott Conservation Park. In the presence of high densities of honeybees, *P. novaehollandiae* underwent a significant alteration in its foraging behaviour and expanded its feeding territories to compensate for a significant decline in nectar availability caused by honeybees foraging at flowers. Paton (1996, 1999) also examined the impacts of commercial hives on the native biota associated with *Banksia ornata* at Ngarkat Conservation Park.

In contrast to the study on *C. rugulosus*, there was no evidence for an impact of honeybees on the native birds, insects or mammals which utilised *B. ornata* nectar. The absence of impacts was attributed to a super-abundance of nectar in *B. ornata* inflorescences, which in turn was attributed to a dearth of native animals in the area.

The impacts of honeybees has also been examined in a number of studies outside Australia. Two studies by Schaffer and coworkers (Schaffer *et al.* 1979, 1983) in Arizona, USA, found that hive honeybees reduced the availability of nectar in patches of *Agave schottii* and led to an alteration in the foraging behaviour of the native bees. The work of David Roubik in the South American tropics has examined the impacts of invading africanised honeybees on native stingless bees. In one study, Roubik (1978) increased the abundance of honeybees by introducing hives to experimental sites, and observed a significant decline in the abundance of native stingless bees and their resource use. In another study, Roubik (1983) experimentally increased honeybee abundance and monitored the brood production and food storage of native social bees. In this study, there was no apparent effect of introducing honeybee hives on the native bees, although it was suggested that the level at which honeybees were introduced to experimental sites may have been too low (around 1 colony per square kilometre) to elicit any response. In a third study, Roubik *et al.* (1986) introduced 20 hives of the African honeybees to a lowland rainforest site in Panama. In the presence of 20 colonies of honeybees, the foraging activity of the native bees declined and the amount of resource harvested by native bees was reduced by around 25%. Roubik *et al.* (1986) concluded that this level of competition could result in the extinction of stingless bee colonies within 10 years.

The effects of forest fragmentation on feral honeybee usage and plant reproduction was investigated by Aizen and Feinsinger (1994a,b) in Chaco forest in Argentina. As the frequency of feral honeybee visits to two native forest species increased from continuous to small forest fragments, visits by native bees, wasps and flies tended to decrease. Finally, a recent study by Murphy and Robertson (2000) in New Zealand examined the impacts of hive bees on native insects foraging on manuka and *Hebe stricta*. The abundance of native flower visitors varied considerably between sites due to variation in weather and site differences. However, the abundance and diversity of native flies appeared to be strongly negatively influenced by honeybees, indicating that the hive bees may have played a role in determining the guild of native pollinators visiting flowers (Murphy and Robertson 2000).

Previous studies on honeybee impacts on native flora

The impact of hive honeybees on seed production by *C. rugulosus* was examined by Paton (1993). Honeybees were found to be capable of pollinating *C. rugulosus* flowers, although less efficiently than the New Holland honeyeaters. Furthermore, honeybees were found to displace honeyeaters from flowers, resulting in a decline in fruit production as the number of honeybees at flowers increased. Paton (1993; also 1996, 1997) also examined the role of honeybees in the pollination of *Correa reflexa* in Flinders Chase National Park, South Australia. Honeybees were found to be less efficient at pollinating flowers than native birds as the former visited mainly recently opened male flowers while birds visited flowers in all stages of anthesis. Furthermore, honeybees may have a negative impact on fruit set in *C. reflexa* flowers by depleting the availability of pollen in male flowers to be picked up and transferred by the legitimate pollinators. A similar phenomenon was described by Wilson and Thomson (1991) where honeybees reduced the amount of pollen in *Impatiens capensis* flowers, leading to reduced quantities of pollen being transferred by native bees.

Paton (1996, 1999) also examined the impact of commercial hives on the seed production in *B. ornata*. In contrast to the previous studies, honeybees appeared to be efficient pollinators of *B. ornata* flowers, with seed production actually increasing significantly in the presence of commercial loads of hive bees. This increase in seed production near apiaries appeared to be due to inadequate numbers of native pollinators (honeyeaters) in the area due to the destruction of adjacent summer and autumn habitat

Finally, Gross and Mackay (1998) examined the impact of introduced hive bees on the reproduction performance of the pioneer shrub *Melastoma affine* in tropical north Queensland. Honeybees were poor pollinators of *M. affine* compared to native bees, and were found to actively strip pollen already deposited on stigmas, resulting in reduced fruit and seed set. Gross and Mackay (1998) concluded that honeybees reduced the fitness *of M. affine* and posed a significant threat to the composition of pioneer species assemblages at rainforest margins.

Honeybee impacts: studies and generalisations

From the previous brief discussion of research into the potential impacts of hive honeybees on native biota, it is clear that the nature, severity and direction of impacts will inevitably be a function of the biological system into which the honeybees are introduced. This in turn leads to four important conclusions

which should be borne in mind when considering the fraught question of the impacts of commercial apiculture in Australia:

(a) First, that obtaining detailed scientific knowledge is an expensive and time consuming process that requires a substantial monetary and time commitment. Studies of honeybee impacts should be carefully designed and include clear hypotheses, robust experimental design and adequate replication, and should ideally cover at least two seasons.

(b) Second, it is important to clearly establish the back-ground level of feral-honeybees, to demonstrate that experimental manipulations do in fact alter honeybee activity at flowers, and to monitor resource (nectar and pollen) levels.

(c) Third, a detailed understanding of the pollination ecology of the native forage species is imperative in interpreting the results of experimental studies. The significance of any perturbations in pollinator activity and pollen flow will be profoundly influenced by the nature and flexibility of the breeding system of a species.

(d) Lastly, different industries, different vegetation types, forage species and seasons, and even different sites will all vary in their biological details and therefore in the likelihood and extent of impacts. Given this contingency of honeybee impacts on specific and local contexts, attempts to generalise the impacts of the introduced honeybee on native animals and plants are both inadvisable and ultimately counterproductive.

Honeybees and apiculture in Tasmania

European honeybees were first introduced into Tasmania in the early 1830's by settlers wishing to produce honey in their new antipodean home. The first hive of English bees (*A. mellifera mellifera*) was set up in what is now Franklin Square, downtown Hobart, in 1831, where it produced between 13-17 swarms in its first season (Parker 1995; Ziegler 1993). The introduction of Italianate honeybees occurred somewhat later in the 1880's (Parker 1995). Feral swarms rapidly spread from managed hives into native vegetation, and by the end of the nineteenth century the honeybee was firmly established as a permanent resident in Tasmania (Ziegler 1993).

The managed production of honey in Tasmania was for many years a local and small scale affair, and the development of a commercial apiculture industry has occurred only gradually over the last 150 years. In the early years there was

a reliance on clover for honey production as nectar sources were easily accessible and the resulting honey was familiar to the palate (Ziegler 1993). The first attempt to utilise the nectar of the Tasmanian leatherwood tree, *Eucryphia lucida* Labill. (Eucryphiaceae), occurred around the 1930's (Parker 1995).

Early attempts to produce leatherwood honey were hampered by bad roads and inadequate vehicles, and regular use of the nectar resource took some time to establish (Ziegler 1993). The production of leatherwood honey increased markedly in the 1970's with improvements in roads and trucks to transport the hives (Ziegler 1993), while the stronger, distinctively flavoured honey also began to be generally appreciated and sought after by consumers. Tasmania currently has over 9000 registered hives producing nearly 650 tonnes of honey per annum, with many more hives and a significant additional production by numerous smaller-scale hobby apiarists (Gibbs and Muirhead 1998). Of this total honey production, over 70% is derived from Tasmanian leatherwood (Ziegler 1993). Commercial honey production in Tasmania makes up *ca.* 2.0% of the national total, and is currently worth around $1.5 million a year to the state (Gibbs and Muirhead 1998).

Study Species: Tasmanian leatherwood Eucryphia lucida

The genus *Eucryphia* is a Gondwanan relic containing six species with a disjunct distribution (Hill 1991). *E. lucida* is endemic to Tasmania and occurs from sea level to 1000 m altitude, while a second endemic Tasmanian species (*E. milliganii*) overlaps the range of *E. lucida* but is more restricted to higher altitudes. Two additional species occur on mainland Australia, *E. moorei* in northern Victoria/southern New South Wales and an undescribed species from a single location in north Queensland (Hill 1991). The two non-Australian members of the genus occur in southern Chile, South America (Hill 1991).

E. lucida is a tall (up to 30 m high) native tree occurring as a canopy co-dominant in cool temperate rainforest in western and southern Tasmania in areas of high rainfall and low fire frequency (Jarman *et al.* 1999; Read 1999). *E. lucida* occurs predominantly in the Thamnic community of cool temperate rainforest as well as in mixed forest with a mature thamnic rainforest understorey, although it may also occur in low abundance in the callindendrous and implicate rainforest communities (Neyland and Hickey 1990; Ziegler 1993; Jarman *et al.* 1999). Approximately 37% of *E. lucida's* distribution lies within the Tasmanian Wilderness World Heritage Area (Ziegler 1993).

Flowering commences in December and lasts for 6-8 weeks, with individual trees bearing thousands of flowers. The four-petalled flowers are white, relatively large (*ca.* 40 mm diameter), actinomorphic and hermaphrodite,

with a central style and 5-7 lobed stigma surrounded by a dense whorl of approximately 80-120 stamens. Each *E. lucida* flower secretes a strong, distinctively flavoured nectar from nectaries at the base of the stamens.

The pollination biology and mating system of *E. lucida* is largely unknown. The only study to date (Ettershank and Ettershank 1992; Ettershank 1993) reported that *E. lucida* flowers are relatively long-lived and protandrous, with a 6-7 day male phase followed by a period of stigma receptivity lasting approximately 6 days. Bagging flowering branches reduced fruit set, although bagged branches still set appreciable quantities of viable seed (Ettershank and Ettershank 1993). *E. lucida* flowers were visited by a broad range of invertebrates covering eight orders of insects and two arachnid orders. Most of these species were nectar and pollen feeders and were considered to be potential pollinators, while the remainder were predators and parasites (Ettershank and Ettershank 1992). The most frequent native insects were tabanid leatherwood flies (*Scaptia* spp.), while feral and hive honeybees were also abundant at flowers (Ettershank and Ettershank 1992).

Previous studies of honeybee impacts on E. lucida

To date, only a single short-term study has addressed the question of honeybee impacts on *E. lucida* and its native pollinators. Ettershank and Ettershank (1992; also Ettershank 1993) compared the numbers of native insects at *E. lucida* flowers at a site near a commercial apiary and at a site around 1 km from the apiary. They noted slightly higher numbers of honeybees near the apiary compared to 1 km away, but found no difference in the numbers of native insects at flowers. Ettershank and Ettershank (1992) concluded that hive honeybees have no impact on the native fauna associated with *E. lucida* flowers, that the *E. lucida* nectar resource is not heavily utilised and is of marginal quality for honeybees, and that current beekeeping practices have no detrimental impacts on the cool temperate rainforest into which hives are introduced.

Aims of the present study

The broad aim of the present study was to obtain detailed information on the native pollinators and pollination ecology of *E. lucida*, and to further investigate the potential impacts of hive honeybees on *E. lucida* and its associated fauna. My specific aims were:

(a) To obtain information on nectar production and consumption in *E. lucida* flowers.
(b) To investigate aspects of the breeding system of *E. lucida*.

(c) To obtain information on the native pollinators of *E. lucida* and their abundance and behaviour at flowers.

(d) To investigate the impacts of hive honeybees on:

1. honeybee activity at *E. lucida* flowers,
2. resource (nectar and pollen) levels,
3. the abundance and activity of native insects at flowers,
4. fruit and seed set by *E. lucida*.

(e) To employ the information from (a), (b) and (c) to interpret evidence from (d) for possible impacts of hive honeybees.

(f) To make management recommendations regarding the likelihood for and amelioration of any detrimental effects of commercial apiculture in the Tasmanian Wilderness World Heritage Area.

Study Sites

I conducted studies at 14 rainforest sites from four locations around Tasmania (Fig. I-V). All sites were in mature stands of thamnic cool temperate rainforest (type T1.1 and T1.2) (Jarman *et al.* 1999), with a canopy dominated by myrtle (*Nothofagus cunninghamii* (Hook. Oerst.), sassafras (*Atherosperma moschatum* Labill.), leatherwood (*E. lucida*), and occasional trees of celery-top pine (*Phyllocladus aspleniifolius* (Labill. Hook. f.), over a dense sub-canopy of horizontal (*Anodopetalum biglandulosum* A. Cunn. ex Hook. f.) and occasional shrubs of native laurel (*Anopterus glandulosus* Labill.) and native plum (*Cenarrhenes nitida* Labill.). Sites were accessed either by road or four-wheel-drive track. *E. lucida* trees tend to flower only when in full or partial sunlight, which in mature rainforest generally occurs in the canopy and adjacent to canopy gaps. For these experiments, I used trees on the edges of roadside and other clearings which flowered to near ground level, and used flowering branches on these trees from 1 m to 2.5 m above the ground. The results of the present study may therefore not necessarily be applicable to flowers higher in the canopy.

One site (MAY) was located 15 km west of Maydena, south-west Tasmania (42° 49′ S 146° 29′ E) (Fig. II). This site was used to examine various aspects of nectar and pollen production and the breeding system of *E. lucida*. MAY was studied in 1999 early in the flowering season (12-15 January; no commercial hives present), in mid season (1-4 February; one small 34-hive apiary within 400 m of site), and in late season (20-22 February; three 34-hive apiaries within 1 km of site), as well as in January and February 2000.

The remaining 13 sites were used to examine the impacts of hive honeybees. Six sites (all *ca.* 600 m a.s.l) were located 5-12 km west of Waratah, north-west Tasmania (Fig. III). Three Waratah sites (WAR1: 41° 27′ S 145° 27′ E, WAR2: 41° 29′ S 145° 31′ E, and WAR3: 41° 29′ S 145° 26′ E) were situated within 400 m of a commercial apiary (50, 60 and 80 hives, respectively), while the other three sites (WAR4: 41° 30′ S 145° 28′ E, WAR5: 41° 32′ S 145° 28′ E, and WAR6: 41° 29′ S 145° 24′ E) were 3 km, 5 km and 2 km from the nearest apiary, respectively. WAR1, WAR2,

WAR4 and WAR5 were studied in February 1998, while WAR3 and WAR6 were studied in February 2000.

Four sites (all *ca.* 300 m a.s.l) were located 22-28 km south of Queenstown on the west coast of Tasmania (Fig. IV). Two Queenstown sites (QT1: 42° 19′ S 145° 36′ E, and QT2: 42° 20′ S 145° 38′ E) were situated within 400 of a commercial apiary (120 and 100 hives, respectively), while the other two sites (QT3: 42° 17′ S 145° 37′ E, and QT4: 42° 21′ S 145° 39′ E) were 3 km and 2 km from the nearest apiary, respectively. QT1 and QT3 were studied in January 1999, and QT2 and QT4 were studied in January 2000.

A further three sites (all *ca.* 250 m a.s.l.) were located along the Western Explorer Link Road, north-west Tasmania (LR1: 41° 28′ S 145° 05′ E, LR2: 41° 29′ S 145° 05′ E, and LR3: 41° 34′ S 145° 05′ E) (Fig. V). The Link Road sites were studied initially in February 1998 when all sites were free of apiaries, and again in February 2000 when two of the sites (LR1 and LR2) had 100 hives, while the third site (LR3) remained free of hives. Site LR3 was 5 km from the nearest apiary site.

Photographs of a number of the sites and of various techniques employed in the study are shown in Figs VI-XIII).

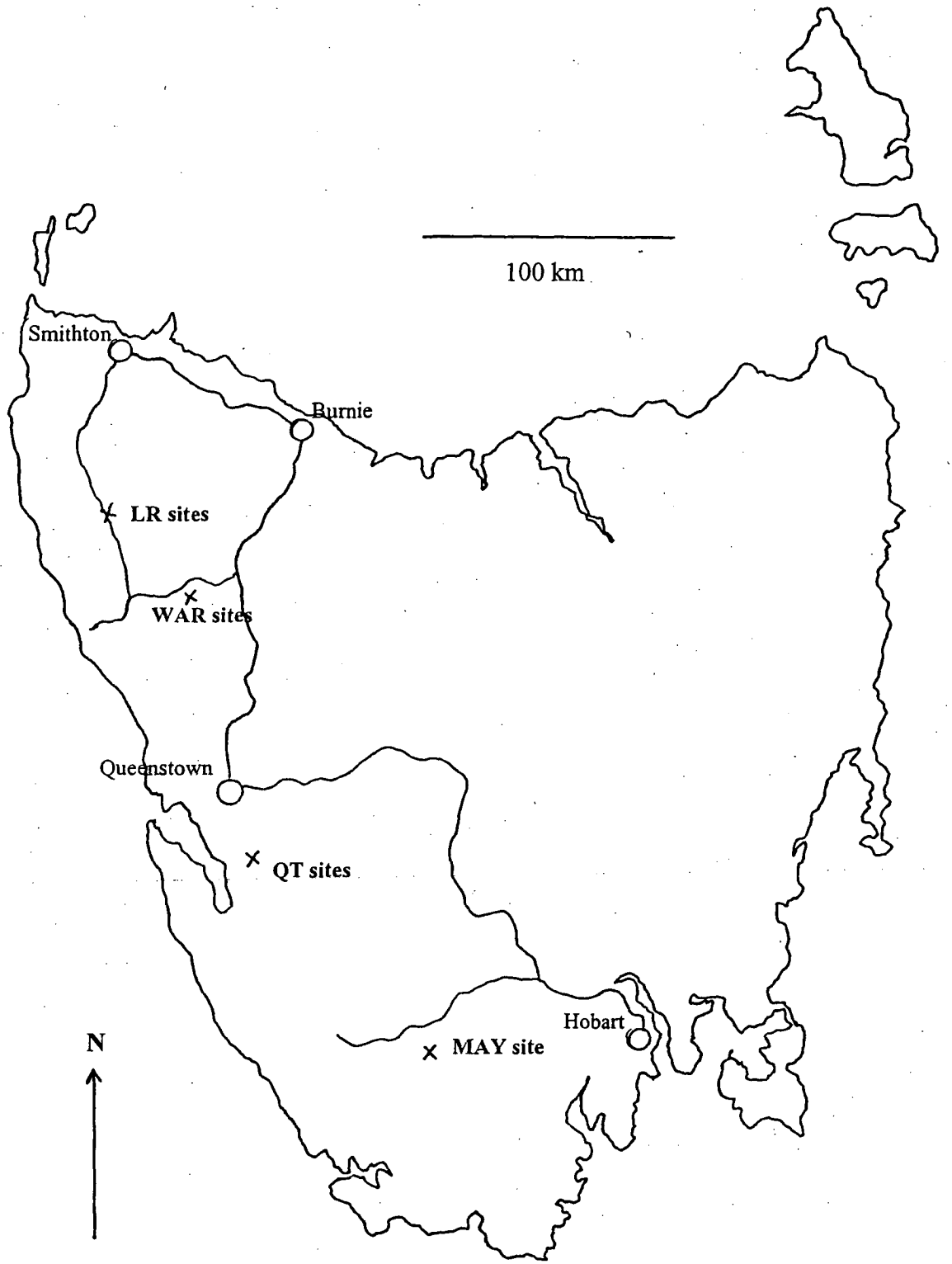

Fig. I. Map of Tasmania, showing approximate locations of the four study areas.

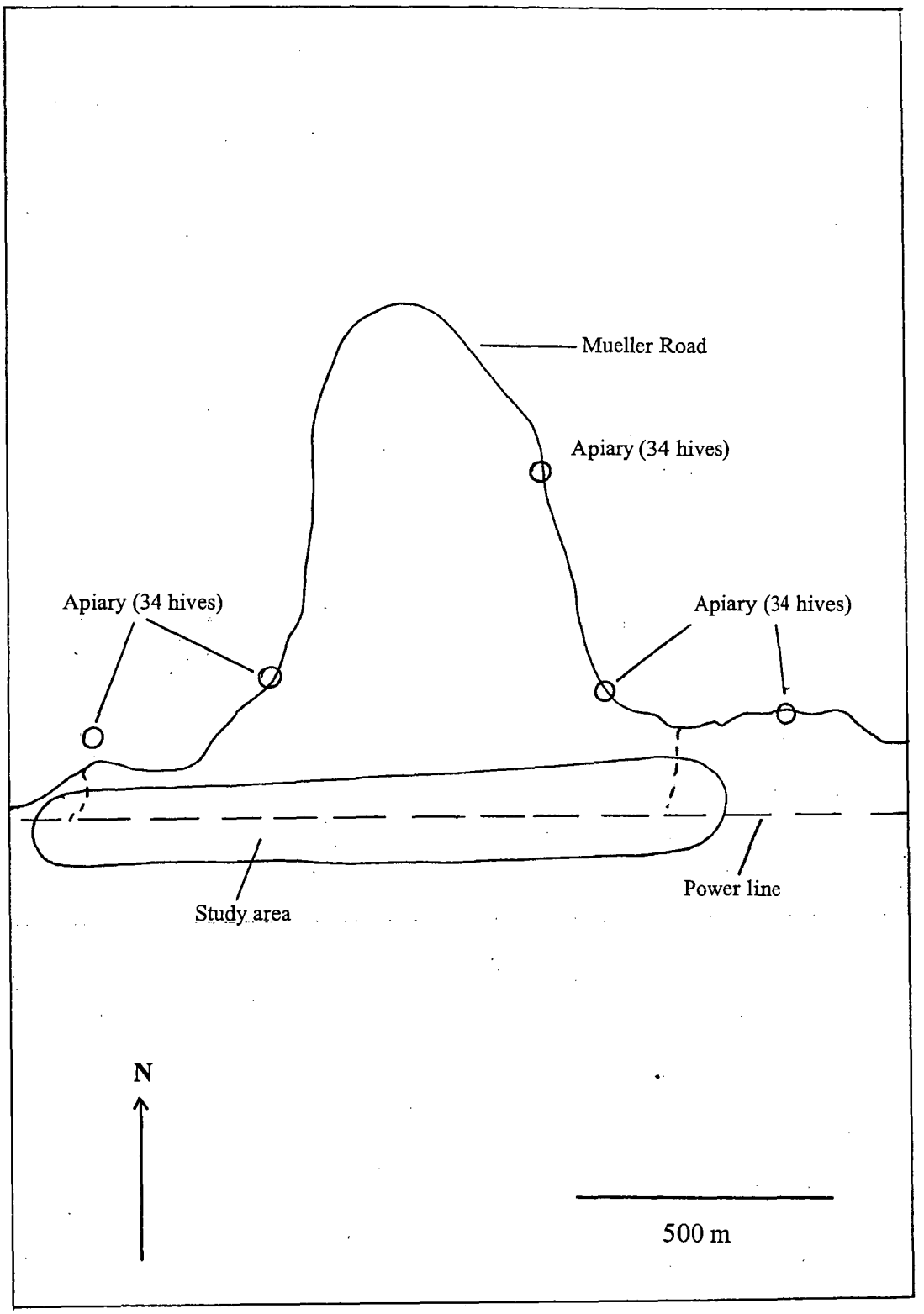

Fig. II. Location of Maydena study site

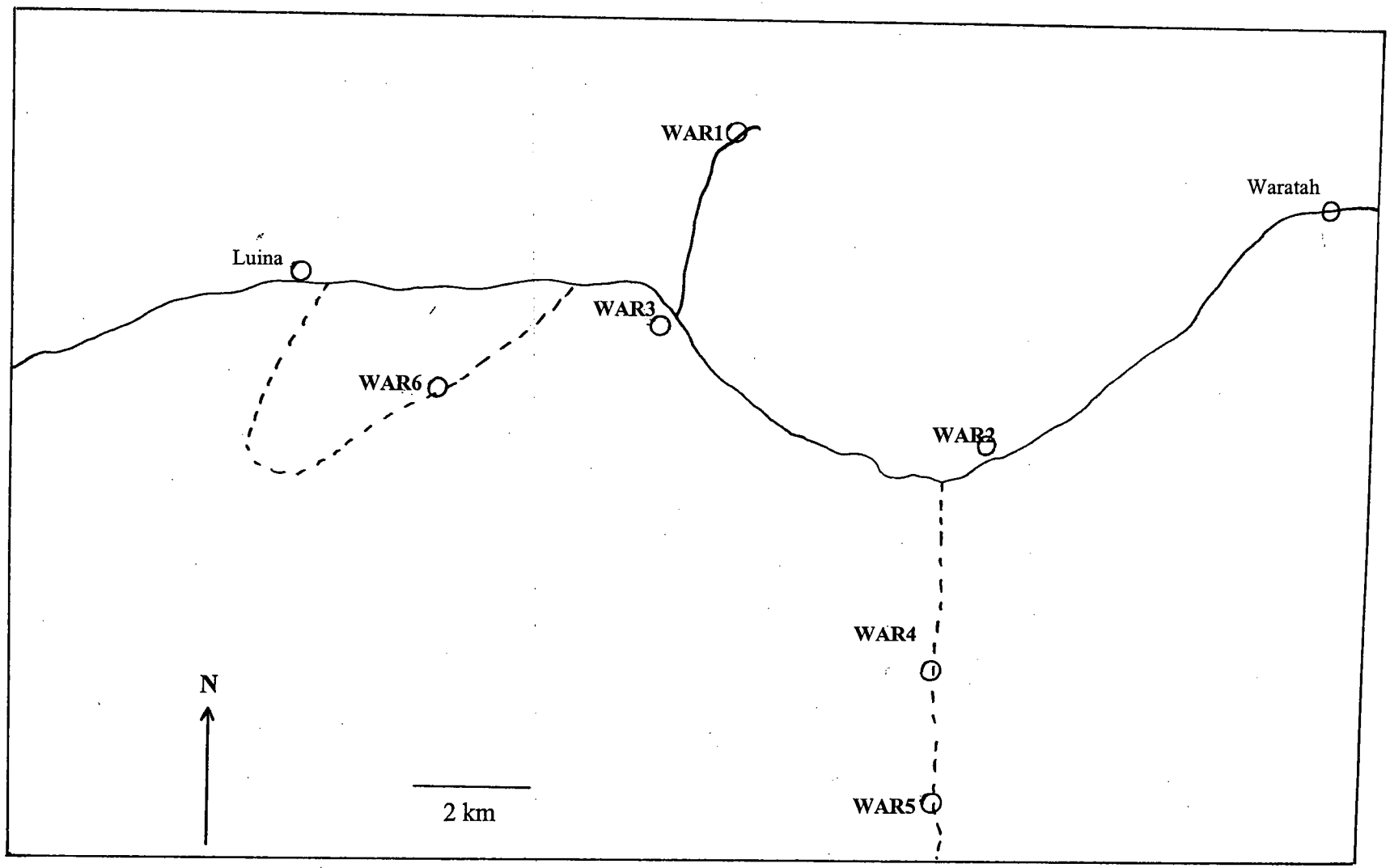

Fig. III. Location of the six Waratah sites.

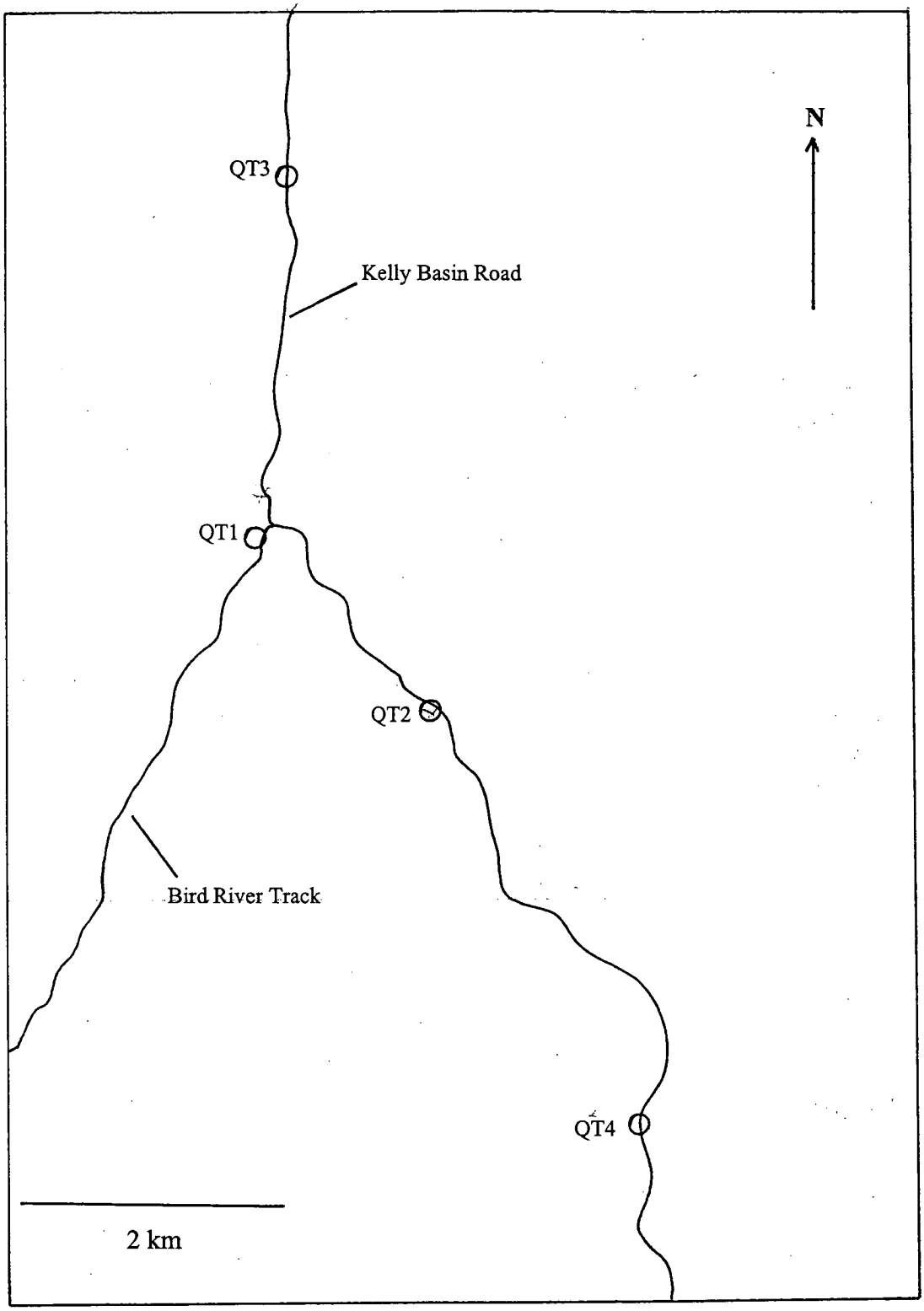

Fig. IV. Location of the four Queenstown sites

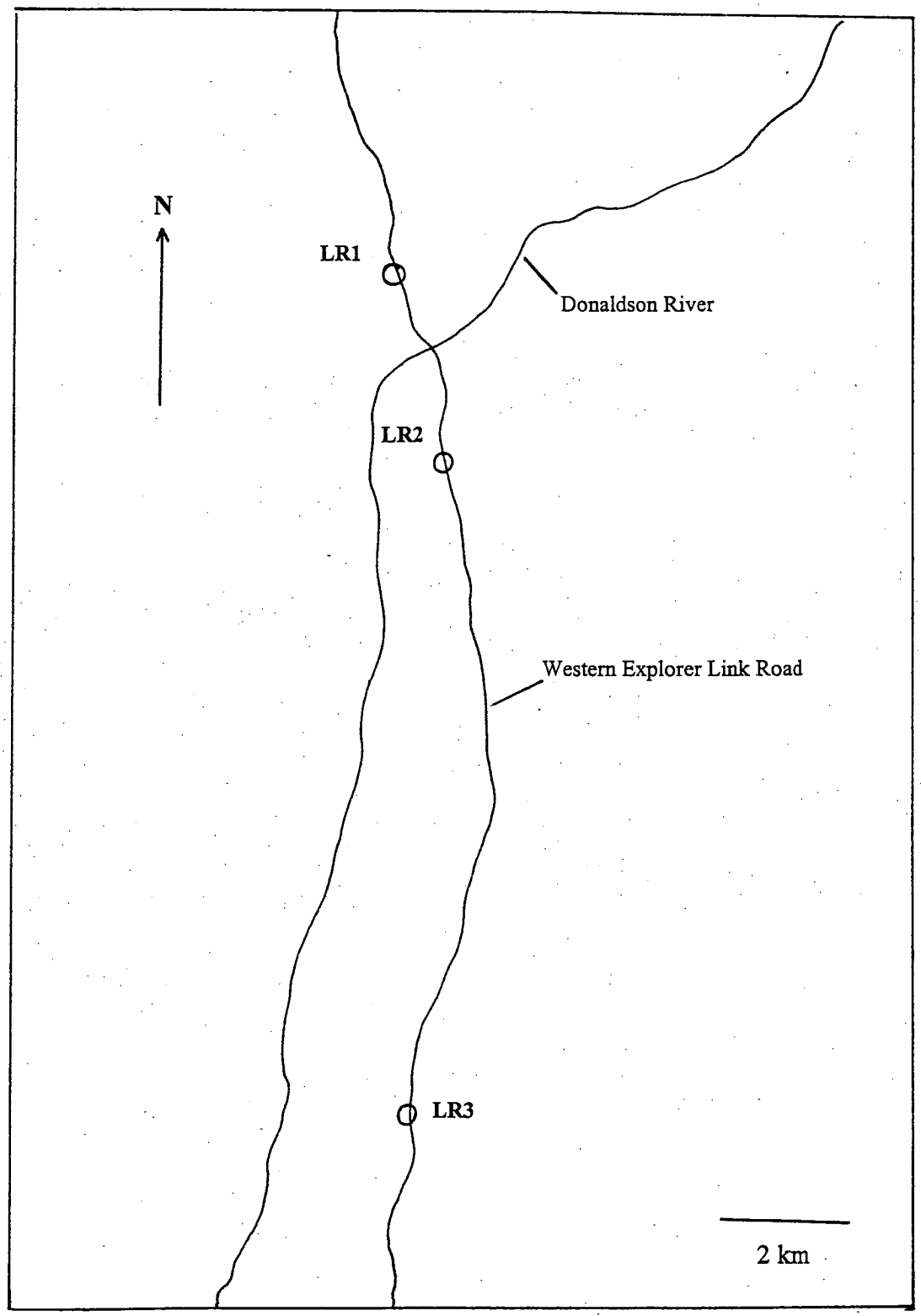

Fig. V. Location of the three Link Road sites.

Fig. VI. Hives at an apiary site at Maydena.

Fig. VII. Maydena study site, showing the power-line clearing with rainforest on either side.

Fig. VIII. Nectar sampling materials, showing refractometer, flowers each with 20 μL of distilled water added to a central pool, and micropipette.

Fig. IX. *E. lucida* flowers of different ages. Top row – young male flowers with all anthers red (un-dehisced); Second row – mid-male flowers with some anthers red, and the remainder either white (pollen still adhering) or brown (pollen dislodged); Third row – female flowers with anthers all brown; Bottom flower – bagged female flower in which all anthers are white (pollen still adhering).

Fig. X. Flowering *E. lucida* tree, with a flowering branch bagged with nylon netting.

Fig. XI. Single E. lucida flower tagged with a loop of coloured wire.

Fig. XII. Video camera in action filming *E. lucida* flowers.

Fig. XIII. Terminal branch of *E. lucida*, showing the fruiting of three years. The top fruit is from the present season with petals just abscissed; the second fruit is from the previous season (still undehisced); while the bottom fruit is from the season before last, with seeds shed.

Part 1. Pollination ecology of *E. lucida*

Chapter 1. Production and consumption of nectar in flowers of *E. lucida*

Abstract

The production and consumption of nectar in *E. lucida* flowers was studied at two locations in Tasmania. Flowers secreted a relatively dilute nectar (*ca* 20% sugar wt/wt). Nectar was produced continuously, although secretion rates were substantially lower at night. At dawn, flowers contained a standing crop of liquid nectar which was rapidly dehydrated on warm days. Due to continuous daytime production, small amounts of concentrated nectar were always present in flowers. This nectar was highly attractive to insects and flowers received multiple insect visitors on warm days. Nectar production was independent of temperature and humidity and was not affected by local shading. Un-bagged flowers on cold days (when insects were inactive) and bagged flowers on warm days rapidly accumulated nectar sugar. There was no evidence for reabsorption of accumulated sugar. These patterns of nectar production in *E. lucida* are interpreted as a mechanism to maximise the frequency of insect visits to flowers in a cool temperate environment in which weather conditions during anthesis can be highly variable.

Introduction

A number of studies have investigated aspects of nectar production in plant species used in the production of honey. However, a complete understanding of the dynamics of nectar production and consumption is so far lacking for any single species (Corbet and Delfosse 1984). One reason for this is the dependence of nectar production on a complex array of environmental variables, including photoperiod, local shading, temperature and humidity, wind speed, soil conditions and the genetic makeup of individual trees (Nunez 1977; Corbet and Delfosse 1984; MacFarland 1985; Harder and Barrett 1992; Wyatt *et al.* 1992; Nicolson 1995). Nectar production may also be strongly influenced by subtle changes in the microclimate around individual flowers (Corbet 1978; Corbet *et al.* 1979; Plowright 1981; Nicolson 1993). The removal of nectar (particularly by insects) is also highly dependent on both the general ambient conditions and the micro-climatic conditions within flowers (Kevan and Baker 1983). A further problem in investigating nectar production is the unknown effect of experimental manipulation on rates of secretion and consumption (Corbet and Delfosse 1984). Removal of nectar from flowers may influence rates of production (Corbet and Willmer 1981; Nicolson 1995), while bagging flowers to exclude visitors may effect the microclimate around the bagged

branch (Corbet and Willmer 1981; Corbet and Delfosse 1984; Wyatt *et al.* 1992).

E. lucida is an important source of nectar in Tasmania's commercial apiculture industry, with over 70% of the state's total honey production derived from this species (Ziegler 1993). Flowering of *E. lucida* begins in early-mid summer, with large numbers of flowers produced over a 4-6 week period (Reid 1989; Ettershank 1993). Flowers of *E. lucida* conform to an open-structured dish- or bowl-shaped blossom (Faegri and van der Pijl 1971). Several dense whorls of stamens arise from a nectar secreting disc at the base of the flower (Curtis and Morris 1981), with the nectar collecting in a sticky mass at the bases of the filaments. Although the stamens may provide some slight degree of physical protection, the secreted nectar is effectively exposed to the external air. *E. lucida* flowers are relatively long-lived, lasting for around 12 days. Anther dehiscence begins soon after flower opening and continues for 4-5 days, with moderate quantities of pollen continuously available in flowers for between 6-7 days. Stigmas become receptive around day 7, and remain so until flower senescence (Ettershank and Ettershank 1992; see Chapter 2). The only study to date on nectar production in *E. lucida* flowers (Ettershank and Ettershank 1992; Ettershank 1993) recorded a dilute (15.6-18.1% sugar wt/wt) nectar in flowers in the early morning in both male and female flowers. Ettershank and Ettershank (1992) were unable to extract liquid nectar after 1000 hours, and concluded that nectar was secreted at night.

In this study I examined aspects of nectar production and consumption in *E. lucida*, including diurnal and nocturnal production rates, diurnal insect visitors to flowers, and the effects of temperature, humidity and local shading on nectar secretion. I also examined whether flowers protected from insect visitors show reabsorption of nectar sugar, and examined the effect of repeated sampling on nectar flow.

Methods

Study sites

Nectar production was studied at WAR1 in 1998 and MAY in 1999, See *Study Sites* section for details of sites.

Choice of days and flowers

Unless otherwise stated, data were collected only on warm, dry days with a temperature maximum of>18°C. All flowers were collected from branches within 3 m of ground level from trees on the edge of roadsides and clearings.

Measuring total floral sugar

Due to the small volumes and the rapid evaporative concentration of nectar, it is often difficult to directly sample nectar in *E. lucida* flowers using micropipettes (cf. Ettershank and Ettershank 1992). In the following experiments, unless otherwise stated I used two rinses with 20μL of distilled water to extract nectar sugar from flowers. Two rinses with 20μL of distilled water removed >95% of total nectar sugar from flowers (see Appendix 1 for details of rinsing method).

Effect of flower age on nectar levels

The age of flowers of *E. lucida* can be estimated from the extent of anther dehiscence; un-dehisced anthers are red, anthers with pollen still adhering are white, while anthers from which pollen has been removed are brown. I identified three flower stages: bud (bud-cap present); male (bud-cap lost, some anthers white, remainder either red or brown); and female (all anthers brown). At MAY on four days in early January, I enclosed one flowering branch with white nylon netting (1.0 mm mesh-size; hereafter referred to as 'bagged') at 0800 hours and measured the quantity of nectar sugar in 10-12 flowers in the bag at 1800 hours using two rinses with 20 μL of distilled water. Each flower was assigned to one of the above stages. All flowers were picked for sampling and then discarded.

Diel patterns in nectar production

Diel patterns in nectar volume and sugar weight per flower were investigated in un-bagged flowers and in flowers bagged since dawn on three clear, warm days at WAR1 in January 1998. Flowers were sampled at 2-hourly intervals from dawn (0600 hours) to dusk (2000 hours). On each day, I selected three trees for sampling and bagged one flowering branch on each sampling tree at dawn. Different trees were used on different days.

At each sampling time, approximately 10 bagged and 10 un-bagged flowers selected from the three trees were picked and initially probed with 5 μL or 20 μL micropipettes to measure the volume of liquid nectar present. Where the volume of nectar removed was sufficient to obtain a refractometer reading (this required >4 μL), the sugar concentration of the sample was measured using a hand-held refractometer (Atago model N1 0-32% and model N2 28-62% sucrose: all measurements adjusted to 20°C). This was followed by two 20 μL rinses with distilled water to extract any remaining sugar in the flower (Appendix 1). Where <4 μL of liquid nectar was extracted, this nectar was blown back into the flower and two 20 μL rinses used to measure total floral sugar. I also examined the diel pattern of sugar weight per flower on a single

cool day (temperature maximum<15°C) at WAR1 in February 1998. Total nectar sugar per flower was measured using two 20 μL rinses in a sample of approximately 10 flowers selected from three trees at 2-hourly intervals from 0800 to 1600 hours. All flowers in these experiments were picked for sampling and then discarded, and different flowers were used on different sampling sessions.

At each sampling time, temperature and humidity were also measured adjacent to a flowering branch using an electronic hygrometer (Templec model no. N19 Y-5189).

Diel Patterns in Insects Visiting Flowers

I used a Sony Handycam Video 8 camera (model no. CCD-TR501E; 15x variable zoom) mounted on a camera tripod (height=1.5 m) to record insects visiting flowers on the three warm days and single cool day used to investigate the production of nectar. For each sampling session, a camera was placed 2-3 m from a flowering tree and trained on a set of 4-10 flowers for a 10-minute segment, after which the camera was moved to a new set of flowers on the same tree for a second 10-minute segment. This was continued for 4 or 5 segments over an hour-long session. Data were recorded on Sony 8 mm (90-minute) cassettes run on long-play mode (i.e. 180 minutes playing time per tape).

Tapes were analysed using a video recorder and TV monitor. For each 10-minute segment, the number of flowers in clear view on the monitor was assessed, and only insect visits to these flowers recorded. For each segment, all floral visits were scored, with visitors classified as either native insects or honeybees. Visit data were expressed as number of visits per flower per 10-minute segment.

Effect of shading, temperature and humidity on nectar production

The effect of local shading, temperature and humidity on the production of nectar was investigated at MAY in early (3 days: 13-15 Jan. 1999) and mid season (3 days: 1-3 Feb. 1999). At 0800 hours, I measured nectar sugar in 10-12 un-bagged flowers from a single tree (flowers were picked for sampling and then discarded), then bagged three flowering branches on the same tree, one with nylon netting, one with a clear-plastic bag (900x900 mm), and one with a double layer of black-plastic bags (600x700 mm). The three bagged branches were situated on one side of the tree with similar aspect. At 1000, 1300 and 1700 hours, I recorded the temperature and humidity adjacent to un-bagged flowers and inside the three types of bag using an electronic hygrometer. At

1800 hours, I measured nectar sugar in a sample of 10-12 flowers from each bag. Six different trees were uesed for baggeing on the six days.

Effect of repeated sampling on nectar production

I investigated the effect of repeated removal of nectar on rates of nectar production. The effect of repeated sampling was investigated over 3 days at MAY in mid season (1-3 Feb. 1999), using the same trees employed in the bagging treatments (above). At 0900 hours, 10 flowers on a single branch were tagged on their pedicel with a short length of coloured wire. Nectar sugar was removed from each tagged flower while still on the tree using two rinses with distilled water, and the flowers bagged with nylon netting. Nectar sugar was then re-measured in these same flowers using two 20 μL rinses with distilled water at 1100, 1300, 1500 and 1700 hours, with flowers re-bagged between sampling times.

Effect of continuous bagging on nectar production

I investigated the effect of several days' continuous bagging on nectar production in flowers at MAY in early season (12-15 Jan. 1999). All flowers in this experiment were picked for sampling and then discarded. Nectar sugar was measured in 10 flowers selected from two trees at 0800 hours (Day 0), and a single flowering branch on each tree bagged with nylon netting. Nectar sugar was measured in 8 bagged flowers from each tree at 0900 hours over the next three days.

I investigated the effect of continuous protection from visitors on nectar production in a second, naturally-occurring experiment. A tree at MAY was observed with many flowers in which the bud cap had failed to release the petals, despite the flower having clearly developed beyond the bud-cap stage. Nectar sugar was measured in a sample of 10 of these flowers.

Nighttime production and consumption of nectar

Nighttime production and removal of nectar was investigated at MAY in early (4 nights: 12-15 Jan. 1999) and mid season (3 nights: 1-3 Feb. 1999). All flowers in this experiment were picked for sampling and then discarded. At 2000 hours, nectar sugar was measured in a sample of 10 (un-bagged) flowers from two trees. A single flowering branch was then bagged on each tree. At 0600 hours on the following day, nectar sugar was measured in 10 un-bagged and 10 bagged flowers from the same two trees. Different trees were used on different nights.

Presentation of data

All data are presented as means ± standard errors.

Results

Effect of Flower Age on Nectar Levels

Flowers of *E. lucida* in bud stage had no nectar. Nectar was first detected in early male flowers as anthers began to dehisce, and continued to be produced until petals started to senesce. Male flowers bagged from 0800 to 1800 hours had slightly more nectar sugar than female flowers (1.02±0.17 mg, n=25 and 0.69±0.19 mg, n=20, respectively), although the difference was not significant (unpaired t-test t_{43}=1.86, P>0.15)

Diel patterns in microclimate, insect activity and floral nectar

On the warm days, temperature increased steadily to a high of 23.7°C at 1400 hours, then declined during the afternoon (Fig. 1.1). Humidity followed the opposite pattern, declining to a minimum of 54% at 1400 hours then increasing again towards evening (Fig. 1.1). Patterns of insect activity at flowers over the three warm days followed the general pattern in temperature and humidity (Fig. 1.2). Both native insects (mainly native flies) and honeybees were rarely observed at flowers before 1200 hours, with most activity occurring between 1200-1600 hours during the warmest, driest part of the day. No insects were observed at flowers after 1800 hours (Fig. 1.2), while no insects were recorded at flowers during the cool day.

In un-bagged flowers, nectar volume was relatively low even in the early morning (0.72 ± 0.07 μL at 0600 hours; Fig. 1.3a). I was able to obtain sufficient nectar (>4 μL) for a refractometer reading from only a small sample of flowers (n=8), all before 1000 hours when conditions were still relatively cool and humid. The mean sugar concentration of this sample was 19.7 ± 1.05% sugar wt/wt, and this is presumed to be close to the concentration of the nectar exudate. Nectar volume in un-bagged flowers declined to<0.1 μL per flower by noon and remained low until late afternoon, then increased again by 2000 hours (Fig. 1.3a). The increase in nectar volume in un-bagged flowers between 1800 and 2000 hours presumably reflects the continuous production of a dilute nectar (Fig. 1.3b) which in the humid early evening remained hydrated (i.e. liquid) and un-consumed by insects. The volume of nectar in bagged flowers varied over the day but was always <1.5 μL per flower (Fig. 1.3a).

In un-bagged flowers on the warm days, nectar sugar declined over the day from 0.51±0.07 mg per flower at dawn to a low of 0.18±0.07 mg/flower by

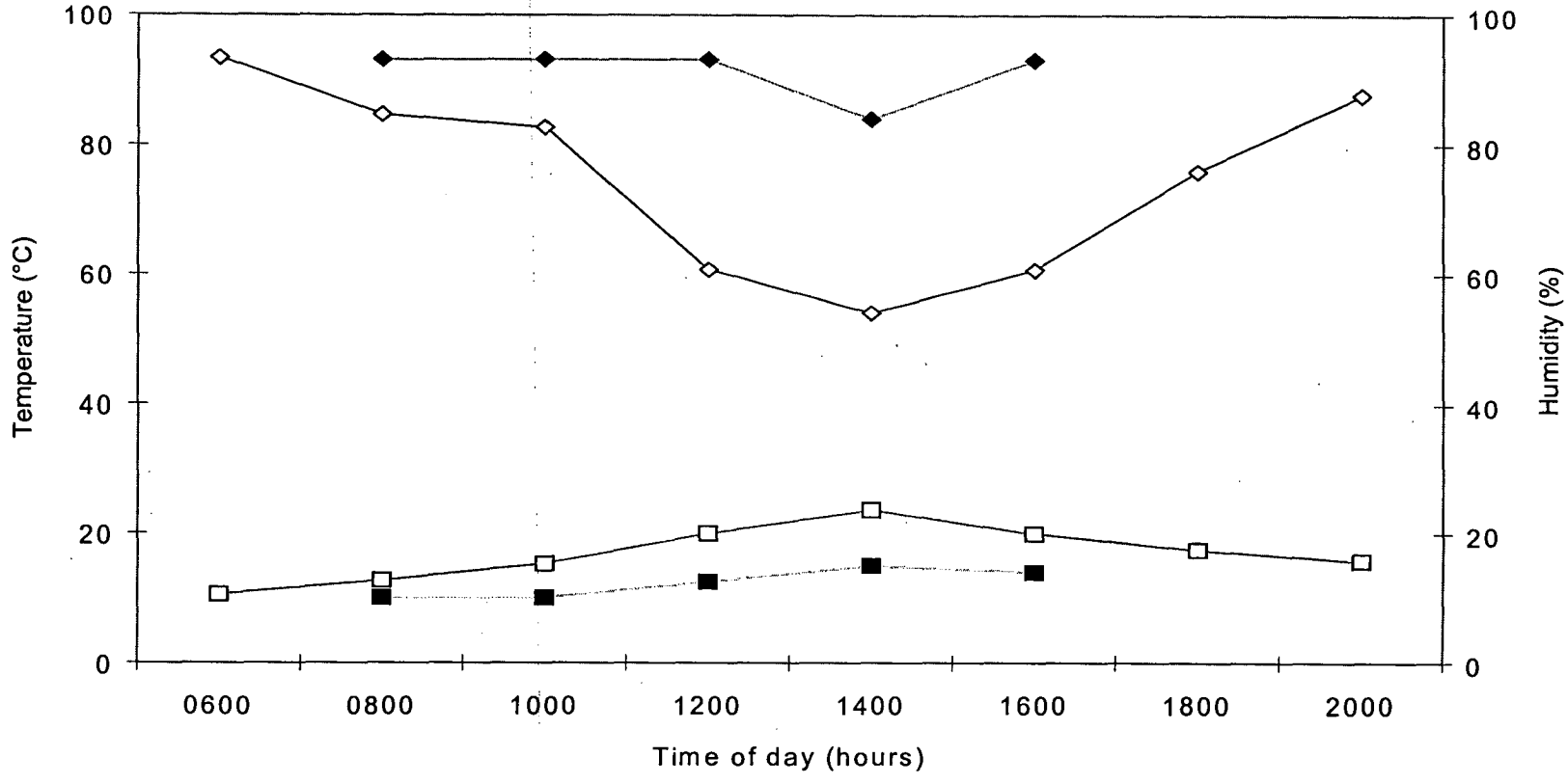

Fig. 1.1. Diel changes in temperature (squares) and humidity (diamonds) on warm days (open symbols: mean of three days) and on a single cool day (closed symbols). n = 12-15 for all points.

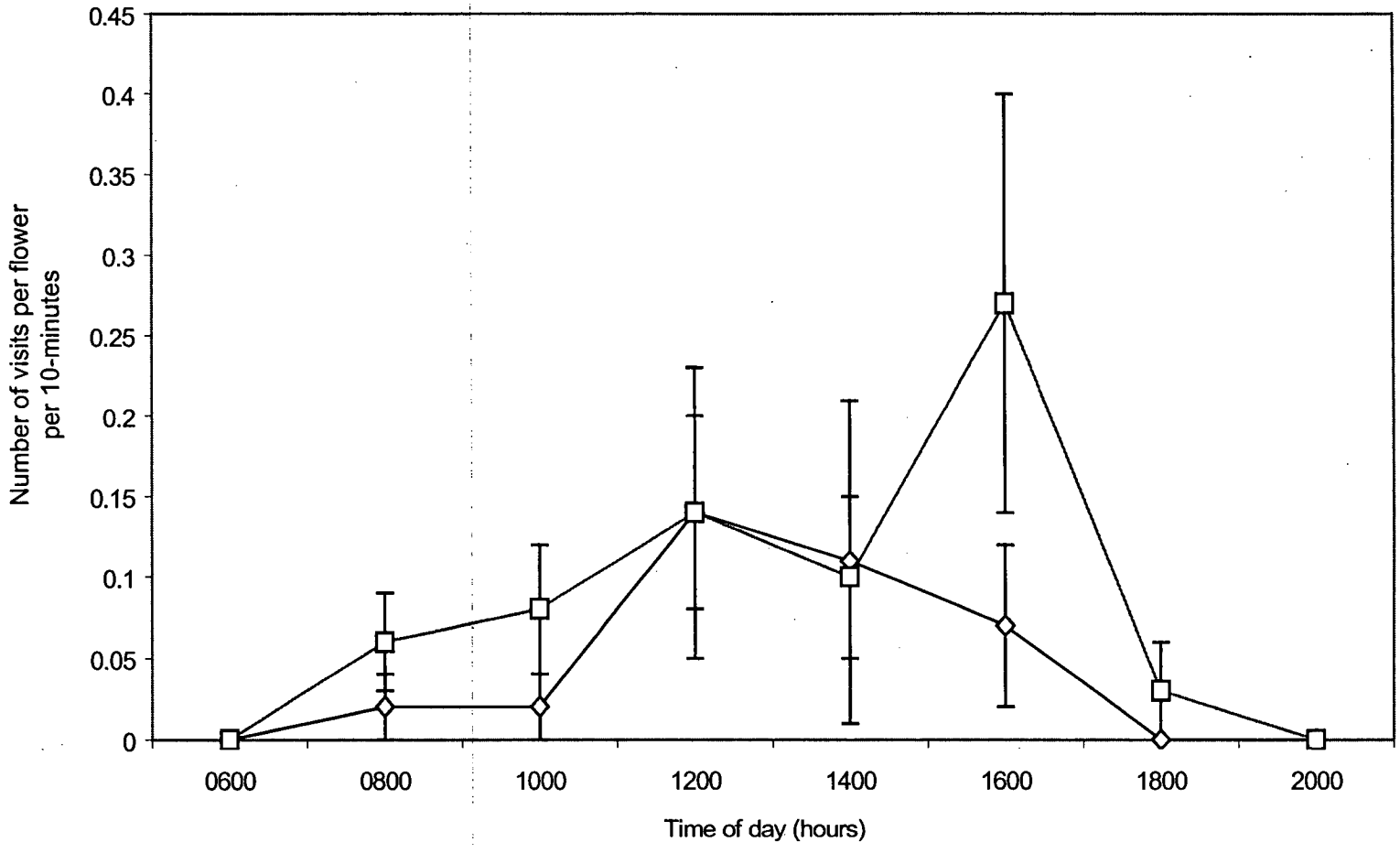

Fig. 1.2. Diel changes in the mean rate of honeybee (diamonds) and native-insect visits (squares) to *E. lucida* flowers over three warm days. n = 12-15 for all points. Error bars are standard errors.

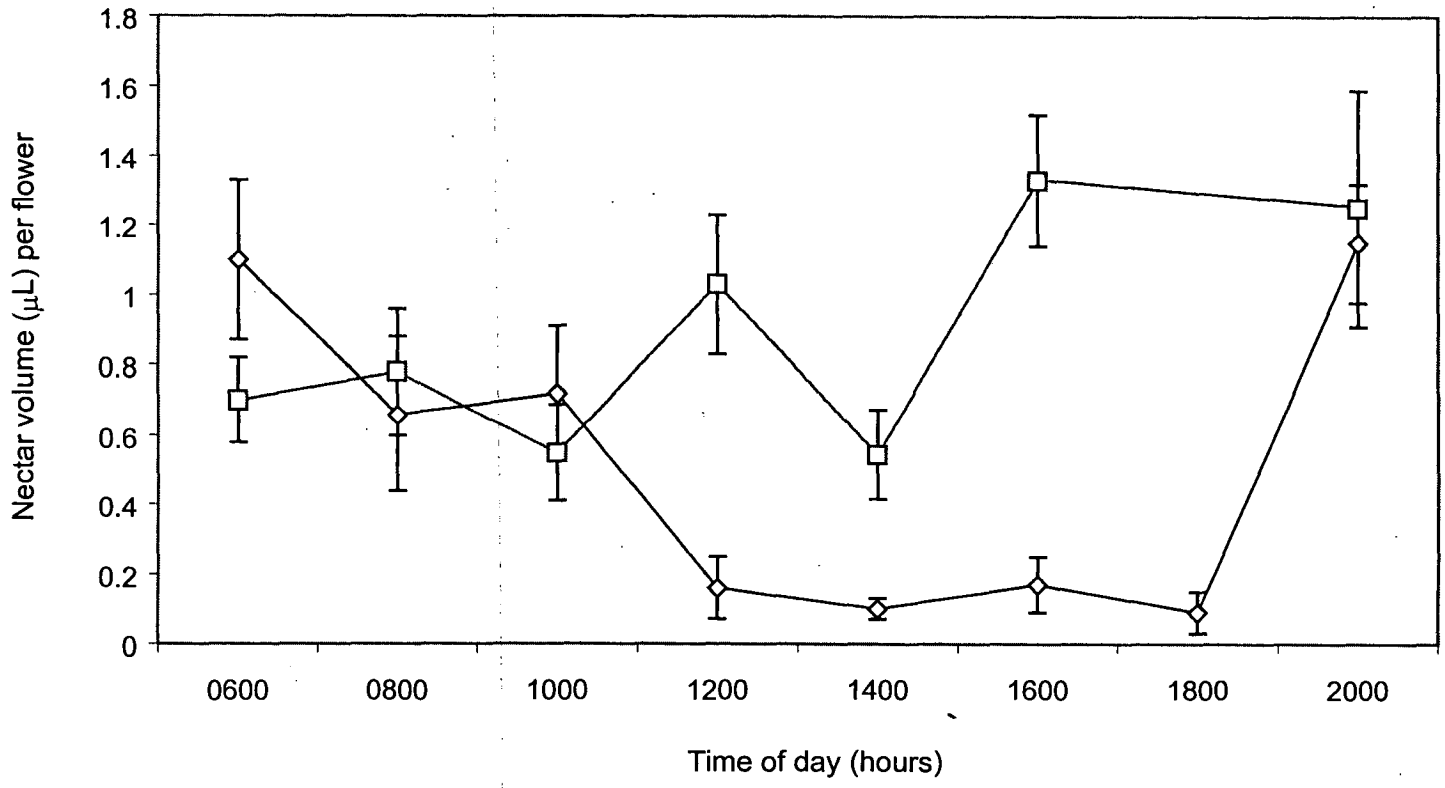

Fig. 1.3a. Diel changes in the mean volume of nectar in *E. lucida* flowers for un-bagged (diamonds) and bagged flowers (squares) over three warm days. n = 15-27 for all points. Error bars are standard errors.

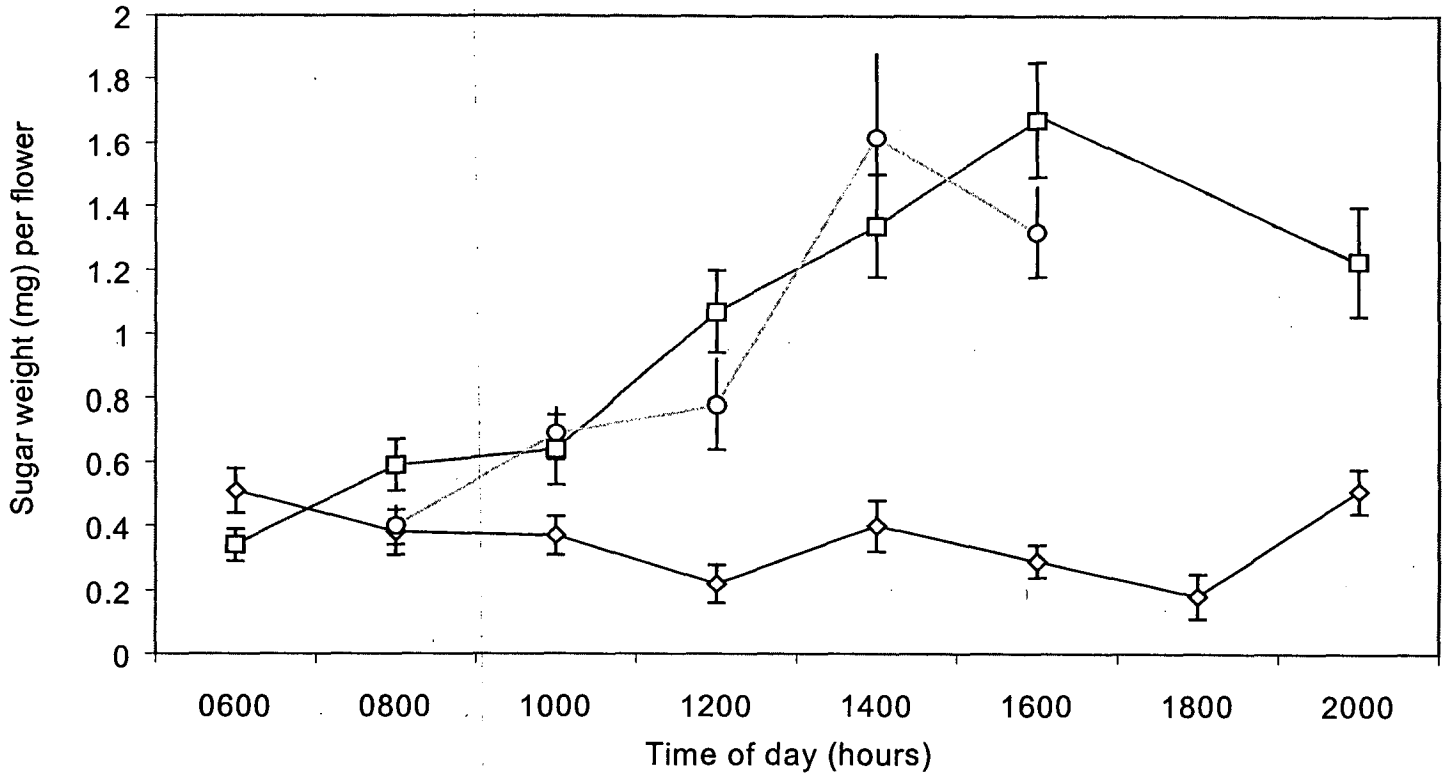

Fig. 1.3b. Diel changes in the mean weight of nectar sugar in *E. lucida* flowers for un-bagged (diamonds) and bagged flowers (squares) over three warm days and for un-bagged flowers on a single cool day (circles). n = 15-27 for all points. Error bars are standard errors.

1800 hours (Fig. 1.3b). After 1800 hours, nectar sugar increased again to close to dawn levels (Fig. 1.3b). In contrast, nectar sugar in bagged flowers increased steadily over the day to a high of 1.68±0.18 mg per flower at 1800 hours, although nectar sugar appeared to decline somewhat by dusk (1.25±0.34 mg/flower). On the single cool day, nectar sugar in un-bagged flowers followed a similar pattern to bagged flowers on warm days (Fig. 1.3b).

Effect of temperature, humidity and shading on nectar production

The mean temperature and humidity at 1000, 1300 and 1700 hours adjacent to open flowers, and inside the three treatment bags (netting, clear plastic and black plastic) are presented in Fig. 1.4a,b. Data for early and mid season are combined. Temperature tended to increase over the day for all treatments; however, temperatures inside both the clear- and black-plastic bags were consistently 2-4°C higher than those inside netting and adjacent to un-bagged flowers (Fig. 1.4a). Relative humidity was consistently close to saturation in the (closed) clear- and black-plastic bags, while relative humidity inside the netting closely followed that of the outside air (Fig. 1.4b). The effect of treatment (including no bag) and time of day on temperature and humidity was tested using a two-way ANOVA. There was a significant effect of treatment and time of day on temperature ($F_{3,56}$=4.10, P<0.025 and $F_{2,56}$=4.10, P<0.001, respectively) and humidity ($F_{3,56}$= 56.7, P<0.001 and $F_{2,56}$=4.99, P<0.025, respectively).

Mean nectar production in the three bagging treatments for the six trees sampled in early and mid season are shown in Fig. 1.5. I tested for an effect of season and treatment on nectar production using a two-way ANOVA on the means for individual trees. There was no effect of season ($F_{1,12}$=1.41, P>0.2), or treatment ($F_{2,12}$=0.09, P>0.5) on nectar production. I also considered the two seasons separately, and tested for an effect of individual trees and treatment on nectar production using a two-way ANOVA. For early season, there was no effect of tree ($F_{1,76}$=1.31, P>0.2) or treatment ($F_{1,7}$ =0.99, P>0.3) on nectar production. For mid season, there was a strong effect of tree ($F_{1,85}$=101.6, P<0.001), due to a single tree with very high nectar (Fig. 1.5), but no effect of treatment ($F_{1,85}$=1.53, P>0.2) on nectar production.

Repeated sampling and nectar production

The mean weight of sugar removed from flowers during repeated 2-hourly sampling is shown in Fig. 1.6. I tested for an effect of tree and time of day using a two-way ANOVA. There were significant differences between trees

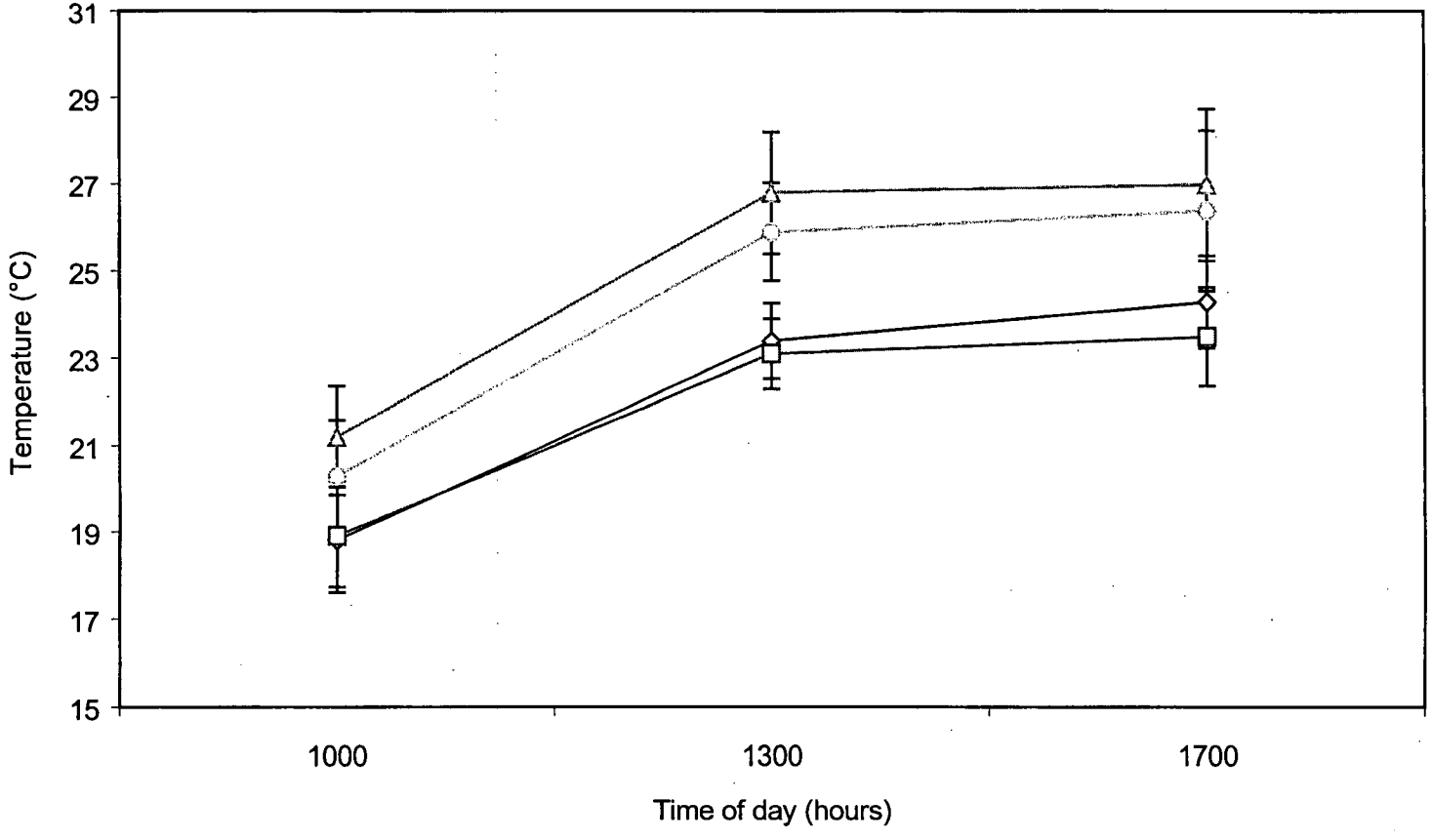

Fig. 1.4a. Mean temperature adjacent to flowers for four flowering-branch treatments. Diamonds: no bag; squares: netting; circles: clear-plastic bag; triangles: black-plastic bag. Error bars are standard errors.

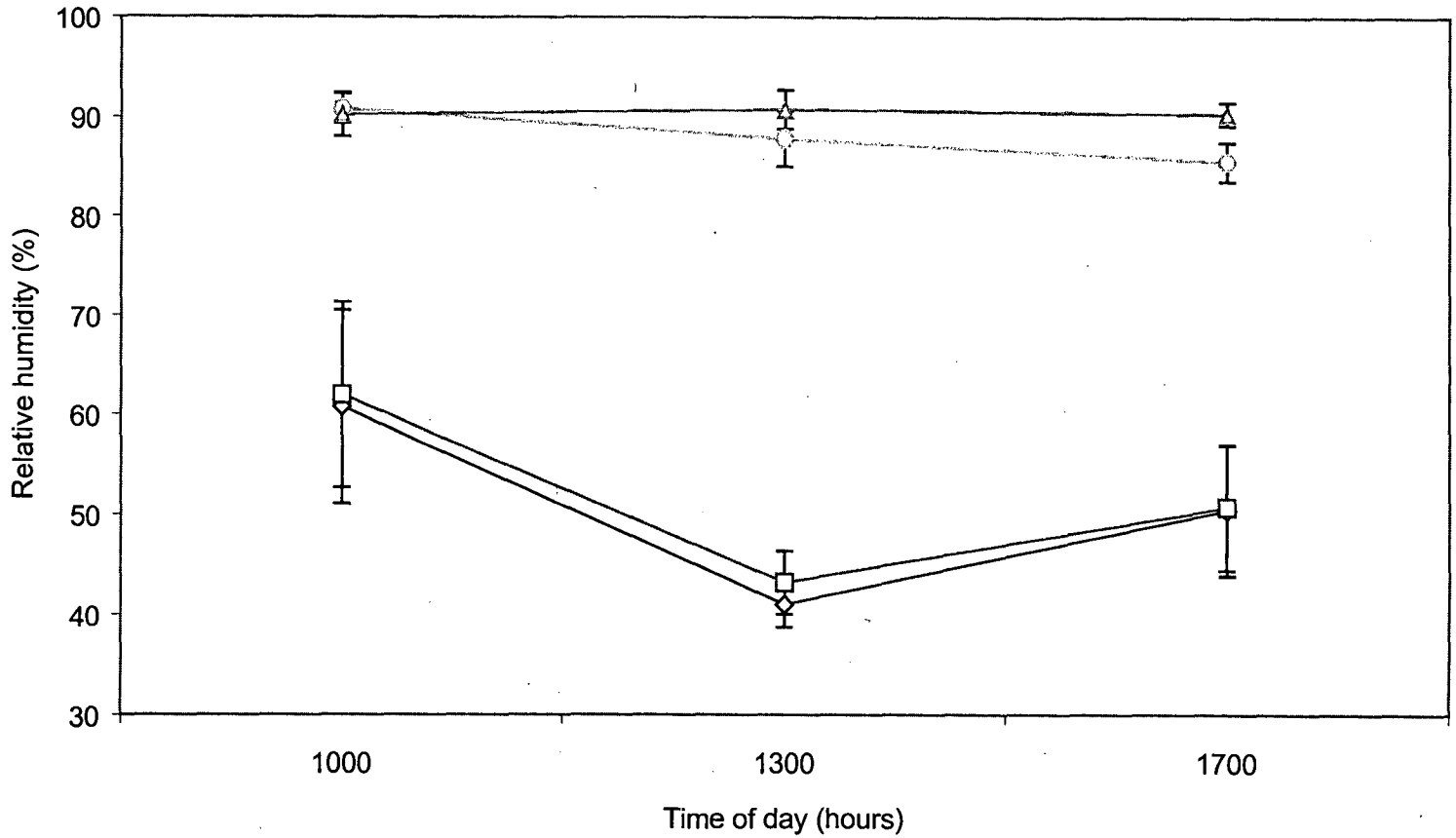

Fig. 1.4b. Mean humidity adjacent to flowers for four flowering-branch treatments. Diamonds: no bag; squares: netting; circles: clear-plastic bag; triangles: black-plastic bag. Error bars are standard errors.

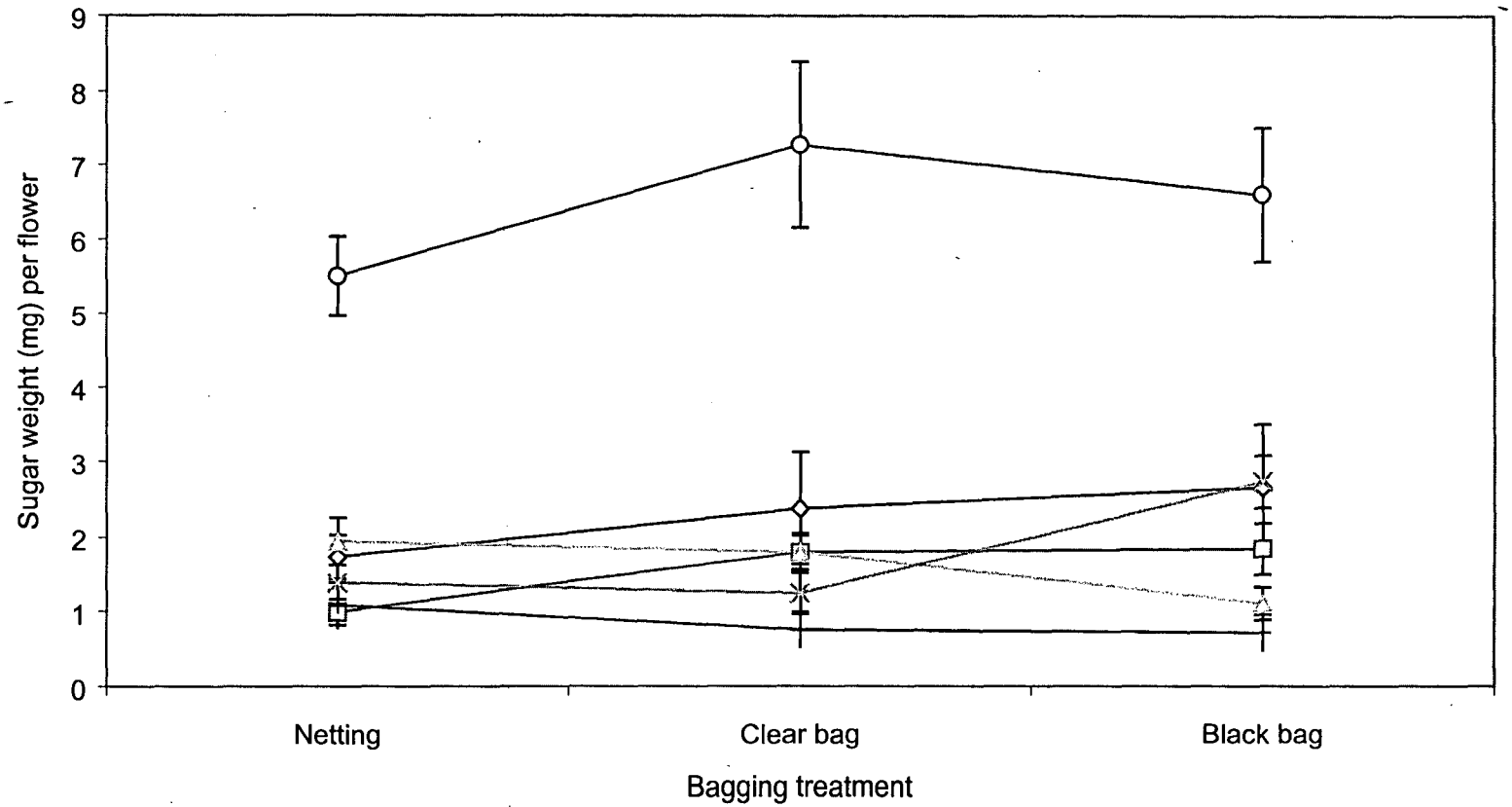

Fig. 1.5. Nectar sugar per flower at 1800 hrs for three flowering-branch treatments. Means for six different trees are shown. n = 8-10 for all points. Error bars are standard errors.

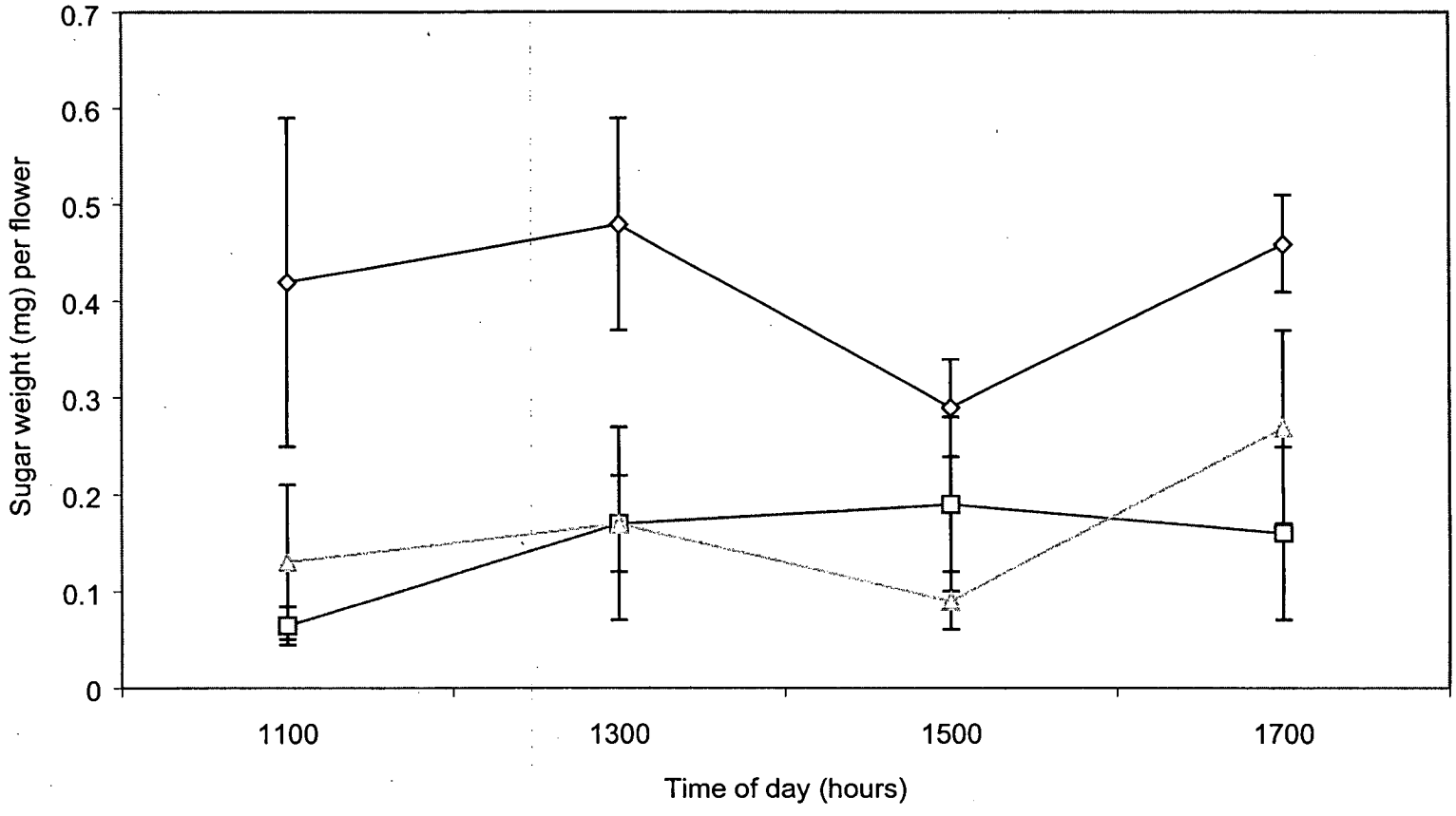

Fig. 1.6. Mean weight of nectar sugar produced by *E. lucida* flowers during repeated 2-hourly sampling. n=8-10 for all points. Three symbols are for three different trees. Error bars are standard errors.

($F_{2,103}$=14.76, P<0.001) but no effect of time of day ($F_{3,103}$=1.40, P>0.2) on nectar production. Because there was no apparent change in the rate of nectar production over the day, I calculated the mean rate of 2-hourly nectar production for Trees 1, 2 and 3 (0.41±0.05, 0.14±0.04 and 0.17±0.03 mg/2-hours, respectively), and multiplied by 5 to give an estimated total nectar sugar production for each tree over a 10-hour day (2.05, 0.72 and 0.83 mg/10-hour day, respectively). These same three trees were used to measure the production of nectar sugar in flowers continuously bagged between 0800 and 1800 hours (see above). Total nectar sugar produced over 10 hours in continuously bagged flowers was 2.08, 0.81 and 0.56 mg/10-hour day for Trees 1, 2 and 3, respectively. The results for the two sampling methods are very similar, and suggests that repeated removal of nectar from flowers did not influence rates of nectar production compared to flowers which remained bagged over the entire day.

Effect of continuous bagging on nectar production

In flowers bagged continuously for three days nectar volume increased initially then reached a plateau after the second day (Fig. 1.7). In contrast, nectar sugar accumulated at an accelerating rate, with increases of 0.99, 1.63 and 4.08 mg nectar sugar on Days 1, 2 and 3, respectively (Fig. 1.7). The concentration of nectar also increased with continuous bagging, with nectar concentrations of 19.60±0.71%, 25.47±0.70% and 45.2±0.94% sugar wt/wt on Days 1, 2 and 3, respectively. By day three, nectar was viscous and difficult to extract with micropipettes.

A single tree at Maydena was observed with many flowers in which the bud cap had not been lost, effectively protecting the nectar from both insect visitors and the external air. These flowers had considerable quantities (mean volume=35.53±15.85 µL, n=8) of a relatively dilute nectar (mean concentration=16.15±1.58% sugar wt/wt), with a mean of 6.07±1.58 mg of sugar per flower.

Nighttime production and consumption of nectar

Nighttime production was determined as the difference between nectar sugar at 2000 hours and nectar sugar in bagged flowers at 0600 hours on the following day, while nighttime consumption was the difference between nectar sugar in bagged and un-bagged flowers at 0600 hours (Table 1.1).

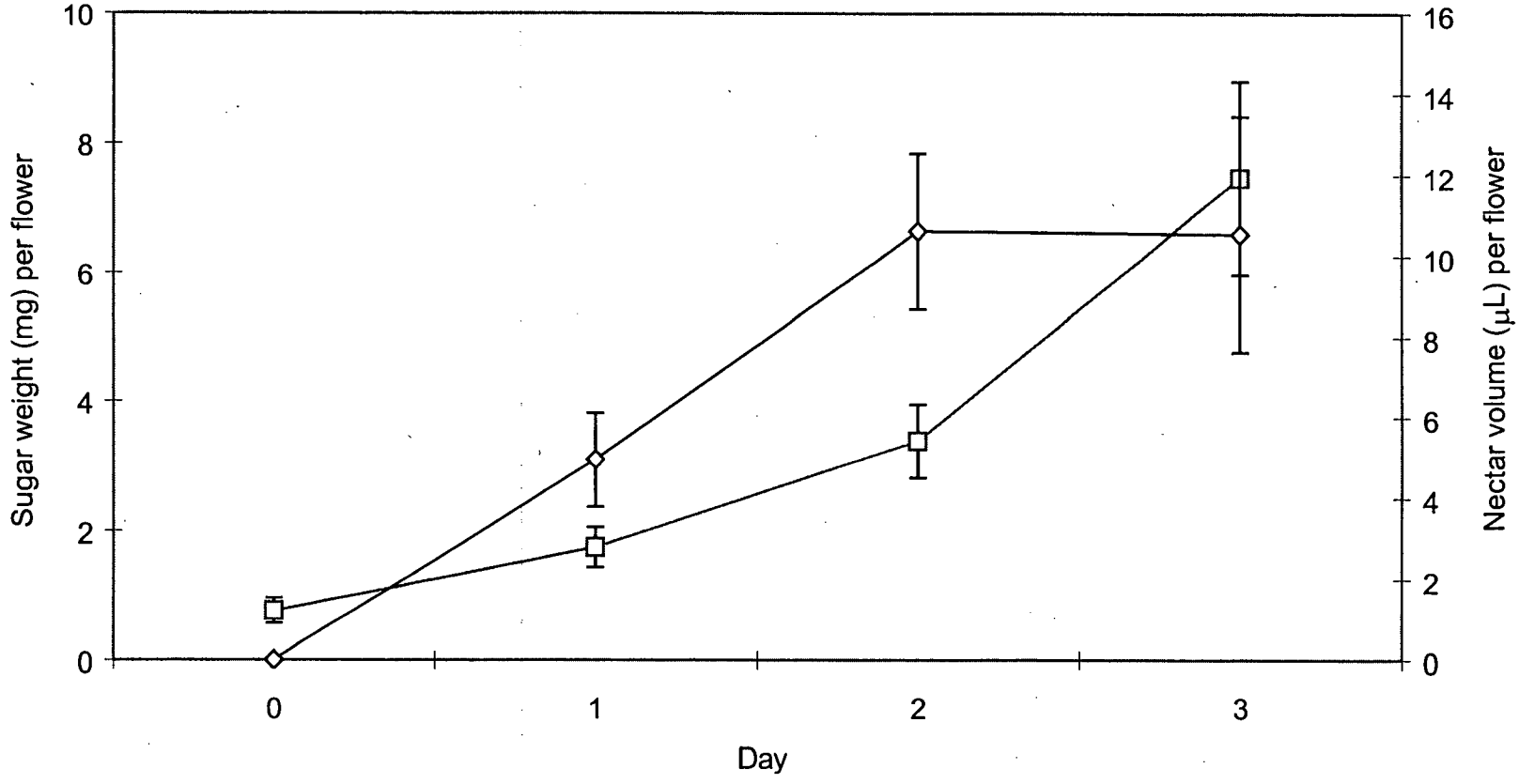

Fig. 1.7. Mean weight of nectar sugar (squares) and nectar volume (diamonds) in *E. lucida* flowers over three days of continuous bagging. n = 9-16 for all points. Error bars are standard errors.

Table 1.1. Sugar weight per flower at dusk (2000 hours) and at dawn (0600 hours) the following morning in bagged and un-bagged flowers in early and mid season. Sample sizes given in brackets.

Season	2000 hours	0600 hours (bagged)	0600 hours (un-bagged)
Early season	0.30 ± 0.04 (64)	0.53 ± 0.05 (64)	0.40 ± 0.05 (6)
Mid season	1.41 ± 0.19 (43)	2.22 ± 0.24 (51)	2.00 ± 0.24 (52)

I tested for a difference between samples using a paired t-test on the means for individual trees. For both early and mid seasons, there was a tendency for nectar sugar in bagged flowers to increase from dusk to dawn (Table 1.1). This difference was significant for both early season (paired t-test, t_7=3.50, P<0.025) and mid season flowers (t_5=3.85, P<0.025), and for the two seasons combined (t_{13}=4.00, P<0.01). The mean quantity of sugar produced over the 10-hour night period was 0.23 mg for early season and 0.81 mg for mid season, respectively (Table 1.1). There was also a tendency for nectar sugar at dawn to be lower in un-bagged flowers compared to bagged flowers (Table 1.1). However, the difference was not significant for either season (early season, paired t-test, t_7=1.28, P>0.2; mid season, t_5=0.76, P>0.4), or for both seasons combined (t_{13}=1.35, P>0.2).

Discussion

The volume, concentration and composition of nectar in a flower are not static phenomena, but alter with changes in rates of secretion and reabsorption and with the post-secretory changes that take place within the flower itself (Corbet 1978; Plowright 1981). The latter, external changes are particularly important in flowers with an open structure in which the physical protection of nectar from external conditions afforded by flowers with a more closed structure (e.g. tubular and bell-shaped flowers) is largely absent (Corbet *et al.* 1979; Nicolson 1994; but see Nicolson 1983).

Flowers of *E. lucida* contained liquid nectar with a concentration of around 20% sugar wt/wt in the early-morning and evening; few insects were active at these times, and this nectar is probably close to the concentration of the secreted exudate (*cf.* Plowright 1981). In un-bagged flowers, nectar volume rapidly declined to <0.1 µL by midday, while nectar sugar per flower declined steadily over the warmest part of the day when insects were most active. In contrast, in flowers protected from insect visitors by nylon netting, there was a steady increase in nectar sugar over the day. However, nectar volume in bagged flowers remained low, indicating the continuously secreted nectar was being

concentrated over the driest part of the day. On warm days, nectar in flowers can exceed 70% sugar wt/wt (see Chapter 3).

Un-bagged flowers held small but appreciable quantities of sugar throughout the day. This sparse but concentrated nectar was clearly attractive to insects, and flowers received multiple visits from both honeybees and native insects. In contrast to bird-pollinated flowers in which nectar is generally around 20-25% concentration, the majority of insect pollinated plants produce flowers with a more concentrated nectar (Faegri and van der Pijl 1971; Kevan and Baker 1983; Wyatt *et al.* 1992). For example, Corbet and co-workers found honeybees and bumblebees preferentially took nectar from flowers of *Sinapis alba* and *Echium vulgare* (Corbet 1978) and *E. plantagineum* (Corbet and Delfosse 1984) where ambient conditions had concentrated nectar to >40%, while flies feeding on nectar of *Crataegus monogyna* also preferred nectar at concentrations of >50% (Corbet *et al.* 1979).

The steady increase in nectar sugar in bagged flowers was also observed in un-bagged flowers sampled on a cool, damp day on which insects were inactive, suggesting nectar secretion is independent of temperature and humidity. This was confirmed by bagging experiments. Nectar produced in flowers bagged with clear plastic, in which temperature and humidity were significantly elevated, was comparable to nectar production in netted flowers in which temperature and humidity were similar to the external air. In contrast to these data for *E. lucida*, nectar production has been shown to be temperature and/or humidity dependent in a number of plant species. In *Gevillea robusta*, an Australian native introduced into South Africa, Nicolson (1995) found nectar production to be strongly temperature dependent up to 30°C. Nunez (1977) found that the flowers of *Eucalyptus melliodora* in Brazil began producing nectar only above 16-18°C, but showed no increase in rate with increasing temperature above this threshold. The secretory activity in *E. melliodora* was related to relative humidity, with a minimum rate of secretion between 1100 and 1600 hours corresponding to the period of low relative humidity (Nunez 1977). Sugar secretion in *E. melliodora* was also reduced when the surrounding atmosphere was saturated, due either to an inhibitory effect of accumulated nectar or by reabsorption of sugar (Nunez 1977).

Nectar is a phloem-sap derivative (Kevan and Baker 1983). Nectar secretion might therefore be expected to be dependent on the photoperiodic circadian cycle, and be effected by local shading during the photophase of the day (Nunez 1977; Corbet and Delfosse 1984). However, I found no evidence for an effect of local shading on nectar production in *E. lucida*. Production in flowers bagged with black plastic was comparable to production in flowers

bagged with clear plastic on the same tree (Fig. 1.5), suggesting sugar was being translocated from other (un-shaded) parts of the tree. Southwick (1984) observed a similar situation in flowers of common milkweed, *Asclepias syriaca*. Nectar production in *A. syriaca* flowers on defoliated stems was not significantly less than foliated stems, with nutrients and carbohydrates apparently passed via an underground rhizome system between neighbouring plants. Similarly, Nunez (1977) found nectar secretion ceased during maximal hours of sunshine in *Eucalyptus melliodora* flowers covered with black cloth, but was reactivated in the late afternoon as the result of far translocation of sugars.

The quantity of nectar sugar in bagged *E. lucida* flowers increased between dusk and dawn, indicating nectar production occurs during the night as well as during daylight hours. However, nighttime nectar production was substantially lower than daytime rates. The total weight of sugar produced per flower in the 10-hour night period was 24.2% and 51.3% of the 10-hour daytime production in early and mid season, respectively. A pattern of sugar depletion during the day followed by overnight replenishment may be typical of plant species visited by diurnal nectarivores (Collins *et al.* 1984). Such a pattern occurs in species in which nectar is produced only during the night, leading to a morning standing crop of nectar which is then removed over the day without being replenished (e.g. *Eucalyptus incrassata* Bond and Brown 1979; *Banksia integrifolia* and *B. spinulosa* MacFarland 1985). However, the same pattern may also occur in species with continuous nectar production where diurnal nectar removal is more rapid than replacement (e.g. *Calothamnus quadrifidus* (Collins *et al.* 1984). Such a pattern appears to occur in *E. lucida* where flowers receive numerous visits from insects, as in the present study, although nectar may also accumulate in flowers during warm days where visitors are rare (see Chapter 8).

A number of studies have found a lower rate of sugar accumulation in continuously bagged flowers compared to flowers which are repeatedly sampled (Corbet and Delfosse 1984; Nicolson 1995). The difference is attributed to the reabsorption of sugar in protected flowers, and is termed 'apparent reabsorption' (Burquez and Corbet 1991). Reabsorbtion of nectar sugar may serve a number of functions, including the retrieval of energetically valuable carbohydrates not being utilised by pollinators (Burquez and Corbet 1991), or the maintenance of constant low nectar concentrations in spite of evaporation (Nicolson 1995).

Repeated removal of nectar from *E. lucida* flowers had no apparent effect on secretory rates, with sugar production in flowers sampled every two hours comparable to production in flowers bagged continuously for a full day. Further evidence that *E. lucida* flowers do not reabsorb nectar came from flowers which

had been protected from visits for several days, both by continuous bagging and in those flowers in which the bud cap failed to release the petals. In flowers bagged continuously for three days, nectar volume increased initially then plateaued at around 10 μL per flower (Fig. 1.7), presumably due to secretion rates equaling rates of evaporative water loss. Nectar concentration also increased (up to *ca.* 45% sugar wt/wt by Day 3), but at a rate substantially slower than that observed in open flowers due to the continuous secretion of dilute (20% sugar wt/wt) nectar which was not being removed by insects. Curiously, nectar sugar appeared to increase over the three days of bagging at an accelerating rate (Fig. 1.7). Such an accumulation in nectar sugar may occur naturally in un-bagged flowers where several cool days (on which insects are inactive) occur in succession.

Nectar production and pollination in E. lucida

Tasmania is dominated year-round by a strong westerly airstream (the 'Roaring Forties'). As a result of exposure to this westerly airstream, areas supporting *E. lucida*-rich cool temperate rainforest in the west and south of the state are heavily subject to intermittent periods of cold and wet weather, with cold periods lasting 1-3 days a common event even in midsummer (Jackson 1999). This climatic variability is accompanied by a concomitant variation in pollinator service as insects are inactive during these cold spells.

Nectar production in *E. lucida* appears to maximise the opportunities for successful pollination in this temporally unpredictable environment. Flowers are long lived (around 12 days), with nectar production commencing immediately after bud opening and continuing throughout anthesis. Despite continual removal of nectar by diurnal insect visitors, continuous nectar production throughout the day ensures flowers always contain small but appreciable quantities of a highly attractive nectar, and flowers receive multiple visits of short duration over a day when weather conditions are warm and dry. Such an extended period of pollen presentation and stigma receptivity accompanied by constant nectar secretion presumably maximises the chances of pollen pickup and deposition during intermittent periods of fine weather (Motten 1983; Beardsell *et al.* 1993; Ashman and Schoen 1994). Multiple visits to flowers by pollinators benefits female function in a flower (i.e. pollen receipt and fruit/seed set) as numerous visits ensure both that pollen is deposited on receptive stigmas, and effectively maximises the chance that a portion of this deposited pollen will be cross pollen (Harder and Thomson 1989). Furthermore, multiple visitors of short duration should also advantage male function (i.e. pollen dispersal) as the proportion of pollen deposited by a visitor is typically a declining function of

the amount removed (i.e. diminishing returns; Harder and Thomson 1989; Thomson *et al.* 1989; Harder and Wilson 1994; see Chapter 5).

E. lucida flowers continue to produce nectar in cold and wet conditions when insects are inactive, ensuring flowers contain substantial quantities of nectar sugar when conditions improve leading to an increase in the duration of insect visits (Thomson and Plowright 1980; Thomson 1986; Klinkhamer and de Jong 1990). However the fitness consequences of an accumulation of nectar and longer visits by insects after cold weather may differ for the male and female functions (Harder and Thomson 1989). Because more pollen is likely to be deposited during longer visits (Thomson and Plowright 1980; Thomson 1986), *E. lucida* flowers with an accumulation of nectar should receive more pollen on stigmas. From the perspective of female fitness, an accumulation of nectar may therefore act to compensate flowers for the absence of insect visits during periods of inclement weather (*cf.* Motten 1983).

In contrast, longer visits are also typically associated with greater pollen removal (Young and Stanton 1990), which would negatively affect male fitness if the proportion of pollen deposited in other flowers declines with the amount removed during a visit. However, such a negative effect on male fitness may be ameliorated in *E. lucida* flowers. Unlike nectar production, the release of pollen (i.e. the rate of anther dehiscence) in *E. lucida* flowers is strongly dependent on temperature, with a lowering of the rate of dehiscence as temperature declines (see Chapter 5). As a result, while flowers with an accumulation of nectar receive longer visits and greater pollen deposition (improving female fitness in female-stage flowers), these same longer visits in male-stage flowers may not necessarily result in larger quantities of pollen being removed and a concomitant reduction in male fitness (see Chapter 5).

Chapter 2. Breeding system of *E. lucida*: mixed mating in a mass-flowering rainforest tree

Abstract

E. lucida is an hermaphroditic, mass flowering canopy tree in Tasmania's cool temperate rainforest. I studied the breeding system of *E. lucida* at MAY in the summers of 1999 and 2000. *E. lucida* flowers were facultatively protandrous: when insect visitors were common, pollen was rapidly removed (flowers completely protandrous), while in flowers protected from insects by bagging, pollen was retained throughout the female phase. Flowers were also weakly herkogamous, and autonomous deposition of self pollen in bagged flowers was substantial. Bagged flowers with a super-abundance of self pollen on stigmas set moderate levels of fruit (34%) with low seed set (16%), indicating substantial abortion of selfed seeds. Un-bagged flowers also received pollen loads well in excess of the number of ovules per ovary, indicating seed set was not limited by the number of grains deposited. Fruit set of un-bagged flowers exceeded 80%, although seed set was relatively low (36%) and highly variable (range 3-85%), presumably due to high levels of geitonogamy. Squatter insects (mainly staphylinid beetles, thrips and spiders) also effected significant levels of fruit and seed set in bagged and emasculated flowers.

Introduction

Plants are said to have a mixed mating system where seed set is a product of both out-crossing and selfing events (Holsinger 1991). The relative selective advantages of cross- and self-fertilisation have received considerable attention over the past 40 years, with earlier theoretical approaches focusing primarily on genetic factors such as inbreeding depression (Lloyd 1979; Lande and Schemske 1985; Charlesworth and Charlesworth 1987) and population structure (Holsinger 1991; Holsinger *et al.* 1984; Steinbachs and Holsinger 1999) in the evolution of mixed mating systems. More recent models, however, have stressed ecological factors as important determinants of the balance between selfing and crossing rates in plants (Lloyd 1979, 1992; Cruden and Lyon 1989; Holsinger 1991; Lloyd and Schoen 1992; Holsinger and Thomson 1994; Barrett and Harder 1996).

Environmental variation in pollinator abundance and activity is an important ecological variable in determining the selective advantages of selfing (Lloyd 1979, 1992). Indeed, since Darwin (1876) first pointed out the advantages of a plant selfing in the absence of pollinators, the phenomenon of 'reproductive assurance' has repeatedly been invoked as the principal advantage

of self-fertilisation, and, more recently, as a powerful selective force in the maintenance of evolutionaryily stable mixed mating systems in plants (Cruden and Lyon 1989; Lloyd 1992; Holsinger 1996).

Of equal importance is the manner in which self-fertilisation is brought about within a flower. In a series of papers, Lloyd (1979, 1992; also Lloyd and Schoen 1992) expanded the conceptualisation of selfing to include a range of selfing modes which differ greatly in their selective value under differing conditions. 'Delayed' selfing (where a period in which cross-fertilisation can occur precedes selfing) is always advantageous, while conditions favoring 'prior' (selfing preceding crossing) and 'competing' selfing (self and cross pollen arriving at the stigma similtaneously) are more stringent (Lloyd 1979, 1992). Lloyd (1992) classified an additional mode of selfing which occurs when environmental conditions are unfavorable for outcrossing ('environmentally induced' selfing) which resembles delayed selfing in being always selectively advantageous.

In contrast to these modes of autogamous (within-flower) selfing, geitonogamy (transfer of self-pollen between flowers of the same plant) has the genetic characteristics of selfing combined with the ecological costs of outcrossing (Lloyd and Schoen 1992; de Jong *et al.* 1993). Geitonogamy may impose fitness costs on both the female (through seed discounting) and male (through pollen discounting) functions of a flower (Barrett and Harder 1996), with recent empirical studies suggesting such fitness costs may be substantial (e.g. Waser and Price 1991; Harder and Barrett 1995). Geitonogamous selfing has particular relevance to mass flowering trees in which the within-tree transfer of self pollen is likely to be very substantial (Carpenter 1976; Augspurger 1980; Hessing 1988; Klinkhamer *et al.* 1989; Klinkhamer and de Jong 1990, 1993).

In this chapter, I describe aspects of the breeding system of *E. lucida*, an hermaphroditic, mass-flowering canopy tree in Tasmania's cool temperate rainforests. I investigated patterns of anthesis, the release, removal and deposition of pollen in flowers, and the ability of *E. lucida* to set fruit and seed under various conditions of pollinator exclusion and flower emasculation. The patterns of pollen removal and deposition and fruit and seed set in *E. lucida* are interpreted in relation to bet-hedging strategies available to mass-flowing plants.

Materials and Methods

Study species and study site

E. lucida is a tall (up to 30 m high) native tree occurring as a canopy co-dominant in cool temperate rainforest in the wetter western and southern regions of Tasmania. Flowering commences in December and lasts for 4-6 weeks, with

individual trees bearing many thousands of flowers. The four-petalled flowers are white, relatively large (*ca.* 40 mm diameter), actinomorphic and hermaphrodite, with a central style and 5-7 lobed stigma surrounded by a dense whorl of approximately 80-120 stamens.

The pollination biology and mating system of *E. lucida* is largely unknown. The only study to date (Ettershank and Ettershank 1992; Ettershank 1993) reported that *E. lucida* flowers are relatively long-lived and protandrous, with a 6-7 day male phase followed by a period of stigma receptivity lasting approximately 6 days. Bagging flowering branches reduced fruit set, although bagged branches still set appreciable quantities of viable seed (Ettershank and Ettershank 1992).

The present study was carried out at MAY in 1999 and 2000. For these experiments, I used trees on the edges of a power-line clearing which flowered to near ground level, and used flowering branches on these trees from 1 m to 2.5 m above the ground.

Pattern of Anthesis

I investigated the pattern of flower-opening and development on a single large flowering branch of a moderately sized tree. The branch was first flagged and the number of developing buds counted in mid-January. The branch was then checked every 3-6 days and the number and developmental stage of all flowers on the branch were counted. I defined three flower phases: bud (bud-cap present), male (some anthers white) and female (all anthers brown). The branch was checked until the final flowers were beginning to senesce (21 days after first check).

Release and removal of pollen

I investigated the pattern of release and removal of pollen in flowers from bud stage to senescence. Pollen release/removal was examined in un-bagged flowers in January and in bagged flowers in early February. For un-bagged flowers, I tagged approximately 150 flowers (spread over 10 trees) in late bud (Day 0) with a loop of colored wire tied to the leaf below each flower. On day three and every second day thereafter, I picked 10-12 flowers, estimated the percentage of white (pollen-bearing) anthers for each flower and removed and mounted stigmas for a count of the number of pollen grains on stigma lobes (method described below). For bagged flowers, I tagged 20 late-bud flowers on two branches and bagged each branch with nylon netting (1 mm mesh size). Flowers were checked on day three and thereafter every two days until day 11, and the percentage of white anthers was estimated for each flower.

Spatial separation of anthers and stigma

Incidental observations of developing flowers indicated that the stigma lobes were positioned at the same level or slightly below the level of the anthers in buds and young (i.e. male) flowers, and that in most (although not all) flowers, the stigma lobes extended out beyond the level of the anthers during the female phase. To quantify the spatial separation of anthers and stigma lobes in female flowers, I used vernier calipers to measure the height (to the nearest 0.2 mm) of the tallest anther (measured from the base of the filaments to the tip of the tallest anther) and the height of the stigma lobes (measured from the base of the ovary to the top of most protruding lobe) in 6-10 female flowers from six trees. The separation of the anthers and stigma lobes was calculated as (stigma-lobe height)-(anther height) for each flower.

Deposition of pollen in un-bagged flowers

To investigate the rate of pollen deposition on stigmas of un-bagged flowers, I tagged approximately 150 flowers (spread over 10 trees) at late-bud stage (day 0) in January. On day one and every second day thereafter, I picked 10-12 flowers, removed the stigma lobes and placed them on a microscope slide in a drop of lacto-phenol blue. Stigmas were left for several minutes to absorb the stain, then were lightly squashed under a coverslip and sealed with clear nail-varnish. The total number of viable pollen grains (those that had absorbed stain; *cf.* Ramsey and Vaughton 2000) and the number of germinating grains (grains in which the beginnings of a pollen tube were visible) were counted under 400x magnification for each stigma lobe for the first three fields of view (one field of view = 448-μm segment of stigma lobe) working down from the stigma tip. Using the mounted stigma lobes, I also obtained a measure of mean stigma-lobe length (distance from lobe tip to junction with style) for 30 stigmas.

Autogamous deposition of pollen in bagged flowers

To investigate the potential for autogamous deposition of pollen in *E. lucida* flowers, I tagged 8 late-bud flowers on a branch in January (total flowers on the branch were approximately n=30), bagged the branch with nylon netting, and liberally doused all flowers with a pyrethrum-based insecticide to kill any 'squatter' insects (small insects using the flower as a semi-permanent haven: Lloyd and Schoen 1992). At the onset of stigma receptivity (Day 7), stigma lobes of tagged flowers were removed, squashed under a coverslip in a drop of lacto-phenol blue and sealed with clear nail-polish. The total number of pollen

grains were counted under 400x magnification for each stigma lobe for the first three fields of view from the stigma tip.

I also examined the number of pollen grains deposited on the stigmas of bagged (plus insecticide) and emasculated flowers. Any pollen deposited in these flowers must have been passively transferred (by wind or water) from the anthers of another flower. Ten flowers on a branch (total flowers on the bagged branch was approximately n=30) were emasculated in late-bud phase, bagged with nylon netting and sprayed with insecticide. After seven days the stigma lobes were excised, mounted and the number of pollen grains counted for the entire stigma lobes.

Fruit and seed set: effect of pollinator exclusions

I investigated fruit and seed set in un-bagged flowers and in flowers exposed to various emasculation/pollinator-exclusion treatments. This experiment was conducted in January 1999 and used 12 different trees, with 15-20 flowers on each tree haphazardly assigned to one the following treatments. Each treatment was identified by a differently colored wire-tag tied to the leaf below each flower:

1. un-bagged flowers, intact (i.e. hermaphrodite)
2. un-bagged flowers, emasculated
3. bagged flowers, intact
4. bagged flowers, intact (plus insecticide)
5. bagged flowers, emasculated
6. bagged flowers, emasculated (plus insecticide)

Flowers in the un-bagged treatments were scattered over the tree, while the bagged flowers were confined to 1-2 branches per tree. These bagged branches had a total of between 30-50 flowers and all bagged branches included both intact and emasculated flowers. The bagged branches were initially bagged while all flowers were still in bud, and were re-bagged after each visit to emasculate and tag flowers. Flowers were emasculated shortly after loss of the bud cap and before dehiscing of anthers had commenced. To emasculate flowers, all anthers were removed by snipping the filaments just under the anther using fine scissors and dislodging the sac from the flower. Due to the staggered opening of flowers on a branch, emasculating the full complement of flowers per treatment (15-20 flowers) required several visits to the branch over a period of 1-2 weeks. The insecticide treatment involved liberally dousing the

treatment branch with a pyrethrum-based insecticide during the first two visits to emasculate flowers.

Once the full complement of flowers had been assigned to each treatment on all trees, the bags were secured with wire collars and left until mid-March when all flowers had senesced, after which bags were removed. All tagged fruits still present on trees were harvested 12 months later (January 2000) shortly before fruits begin to dry out and open (Read 1989). Fruits were left in open plastic containers at room temperature until the seed capsules started to split. All dried fruits were weighed to the nearest 0.001 g before seeds were removed and counted. I also recorded the number of seed capsules per fruit. The number of developed seeds (fully expanded) and undeveloped seeds (small or only slightly expanded) were then counted for all those fruits in which the seed capsules dehisced fully. A number of fruits (particularly from the bagging treatments) failed to dehisce completely. For these partially opened fruits, the developed seeds could be removed and counted by prizing open the seed capsules with forceps, but it was impossible to accurately count the undeveloped seeds which tended to shatter when the capsules were forced. A portion of fruits failed to open at all

I assumed that the total number of ovules per ovary equaled the sum of the developed and undeveloped seeds in the fruits. Percentage seed set was therefore (developed seeds/[developed+undeveloped seeds])*100. However, I could not directly measure this for fruits which did not fully dehisce as for these fruits I had no count of undeveloped seeds. To estimate seed set in these partially dehisced fruits, I first calculated the mean number of ovules per seed capsule using data from the fully-dehisced fruits (mean ovules/seed capsule=6.83±0.09, n=231). For each partially-dehisced fruit, I then estimated the total ovules per ovary as the mean ovules per capsule*number of capsules for each fruit, and calculated percentage seed set as above.

I tested for an effect of treatment on percentage fruit set, percentage fruit fully/partially opened, percentage seed set, and fruit weight using a random block ANOVA, with trees and treatment as random and fixed factors, respectively. The percentage data were arcsine transformed, and all data were natural log-transformed to improve normality. Post-hoc pairwise comparisons of means used the Bonferroni test.

Results

Pattern of anthesis

Flowering of individual *E. lucida* trees at the study site was staggered to some degree, with early trees first breaking into flower in late December. By mid-

January, most trees were in heavy flower. Flowering trees were present throughout January and February, although only occasional trees still bore flowers into March. The flowering of branches on individual trees was also not fully synchronous, with some branches still coming into bud up to four weeks after flowers first appeared on the earliest-flowering branches.

The single branch on which flowering was regularly monitored had a total of 54 buds when first visited in mid-January, although additional buds appeared during the monitoring period (Fig. 2.1). There was substantial overlap of the male and female flower-phases, although initially there were only bud and male flowers present, while toward the end of the branch's flowering period the great majority of flowers were female (Fig. 2.1). Generally speaking, individual *E. lucida* trees carried both male and female flowers throughout their flowering period, with only a very brief period during initial (all male) and final (all female) flowering when trees were effectively unisexual.

Release and removal of pollen

Dehiscing of anthers commenced soon after the loss of the bud cap and continued for 4-5 days in a staggered fashion, with the inner anthers (close to the stigma) tending to dehisce earlier than those in the outer whorls. In un-bagged flowers, the percentage of anthers bearing pollen increased over the first five days of a flower's life, although no more than 20% of anthers carried pollen at any one time (Fig. 2.2). After day 5, the amount of pollen in flowers rapidly declined, with >98% of pollen removed from flowers by day 7 (Fig. 2.2). In bagged flowers which were protected from insect visits, the percentage of anthers bearing pollen increased steadily until day 7 when over 60% of anthers were coated with pollen, after which the number of pollen-bearing anthers declined (Fig. 2.2). However, >15% of anthers still carried pollen after 11 days by which time the filaments had started to wither (Fig. 2.2).

Spatial separation of anthers and stigma and stigma-lobe length

There were significant differences in the separation of anthers and stigmas in female flowers between the six trees (one-way ANOVA, $F_{5,34}=19.42$, $P<0.001$). On one tree, the stigma lobes of female flowers were positioned below the anthers (mean separation= -1.78 ± 0.27 mm), while for the remaining five trees stigma lobes ranged from close to level with to several mm above the level of the anthers (mean range of separation: 0.07 ± 0.25 - 2.16 ± 0.28 mm). The mean length of stigma lobes was 3.66 ± 0.12 mm (n=30; range=2.4-4.7 mm). Therefore even in those female flowers with maximum separation of anthers and stigma, there was some overlap between anthers and the receptive stigma lobes.

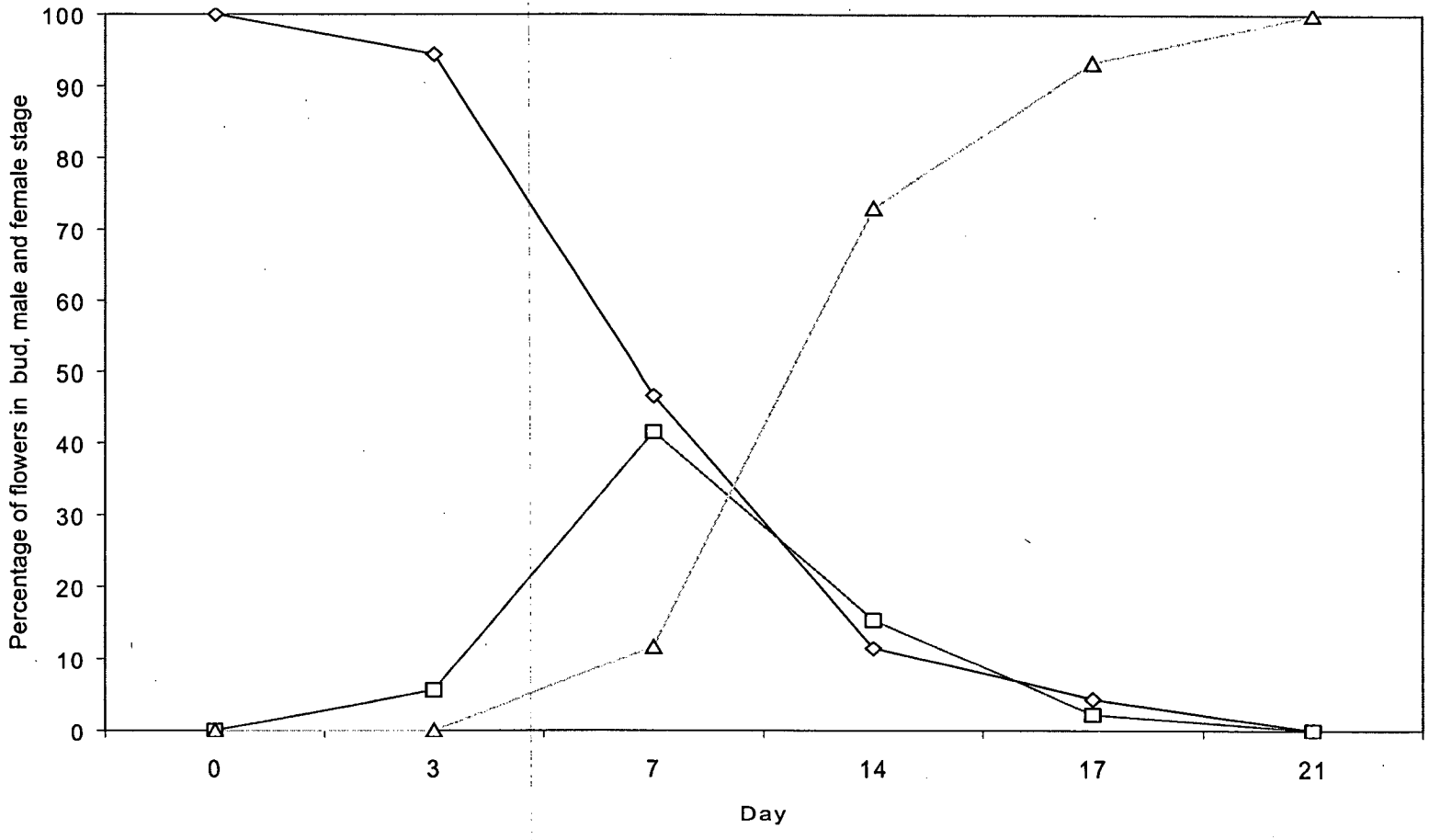

Fig. 2.1. Pattern of anthesis on a single *E. lucida* branch, showing proportions of flowers in bud (diamonds), male (squares) and female (triangles) stages.

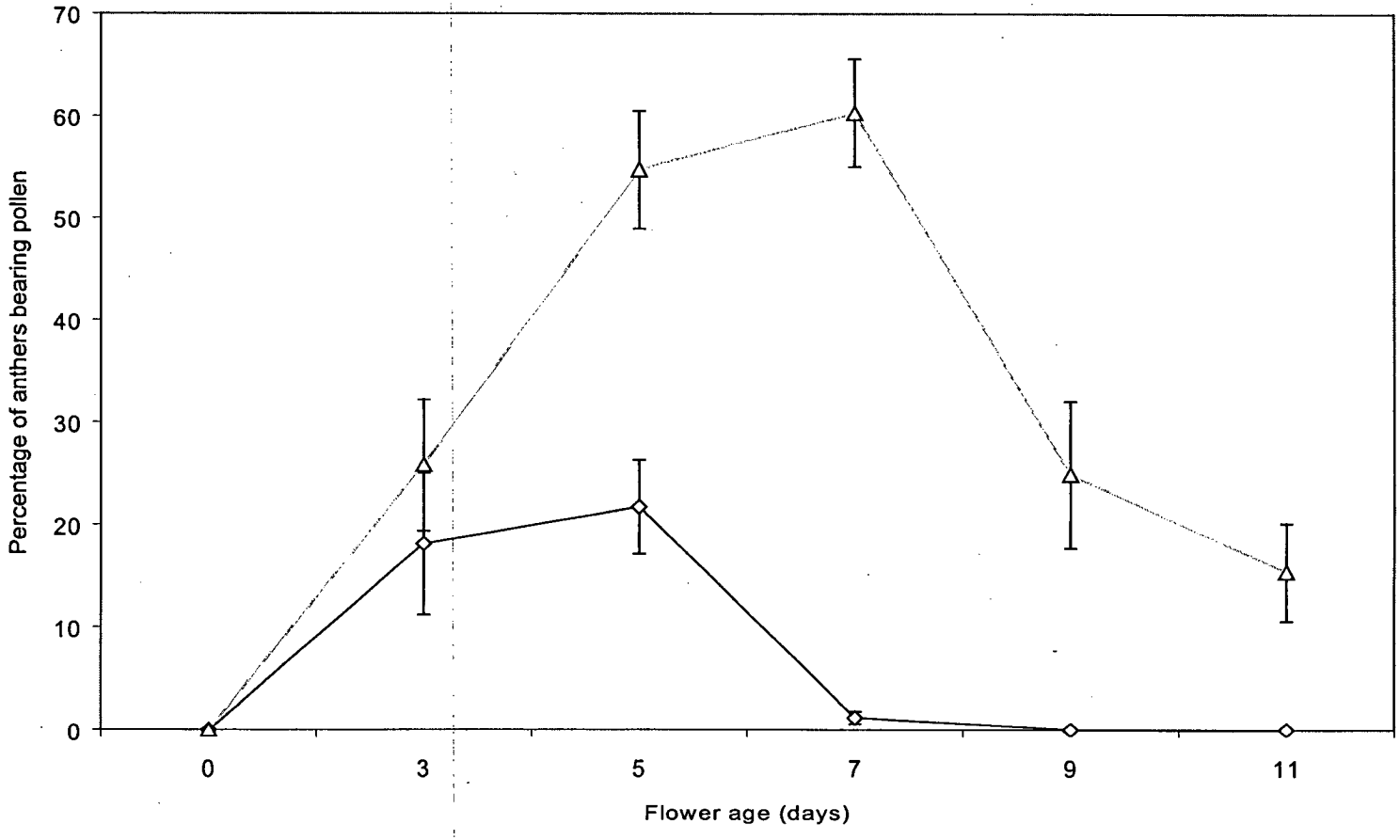

Fig. 2.2. Percentage of anthers bearing pollen over the lifetime of a flower in un-bagged flowers (diamonds) and in bagged flowers (triangles). n=10-20 flowers for all points. Error bars are standard erors.

Deposition of pollen in un-bagged flowers

Stigmas of un-bagged flowers had pollen grains adhering throughout anthesis (Fig. 2.3). Germinating grains were first observed on stigmas of 7-day flowers (2.1±2.1% of grains germinating) and thereafter on 9-, 11- and 13-day flowers (13.3±5.0%, 18.5±2.5% and 9.6±5.3% of grains germinating, respectively), indicating stigma receptivity commenced around day 6-7 and persisted until flower senescence. The number of viable pollen grains on the first three sections of stigma lobes varied over the lifetime of a flower, particularly during the first 5 days of anthesis (Fig. 2.3). For the entire lifetime of the flower, there was no effect of stigma-lobe segment ($F_{2,186}$=1.0, P>0.3) but a strong effect of flower age ($F_{6,189}$=4.4, P<0.001) on the number of grains (two-way ANOVA on natural-log transformed data). For the male phase (days 1,3 and 5), there was no effect of segment ($F_{2,78}$=0.1, P>0.5) but a significant effect of flower age ($F_{2,78}$=5.6, P<0.01) on the number of pollen grains. However, for female flowers (days 7,9,11 and 13), there was no effect of either stigma-lobe segment or flower age on the number of viable pollen grains ($F_{2,108}$=1.6, P>0.2 and $F_{3,108}$=1.0, P>0.4, respectively).

These data suggest that pollen grains are deposited in similar numbers along the length of stigma lobes (i.e. no effect of stigma section), and that during the female phase, germinating grains were continually being replaced by incoming pollen (i.e. no effect of day). The mean number of pollen grains per lobe-segment per day during the female phase was 4.82±0.49 (n=120) (Fig. 2.3). The mean length of stigma lobes was 3.66±0.12 mm (n=30), so there were approximately eight segments (each 448-μm in length) per lobe. The mean number of stigma-lobes per flower was 6.4±0.05 (n=262). Therefore, the total number of pollen grains deposited on the entire stigma of a flower during the female phase can be estimated as:

The mean number of grains/lobe-segment/day (4.82±0.49)*number of segments/lobe(8)*number of lobes/stigma (6.4±0.05)*number of 'female' days (7) = 1727.5 grains.

However, this is likely to be an upper estimate as not all days are likely to be suitable for insect visits due to poor weather.

Autogamous deposition of pollen in bagged flowers

Stigmas of intact flowers that had been bagged since bud for seven days had substantial pollen loads (mean grains/448-μm segment=16.5±5.1, n=8). This

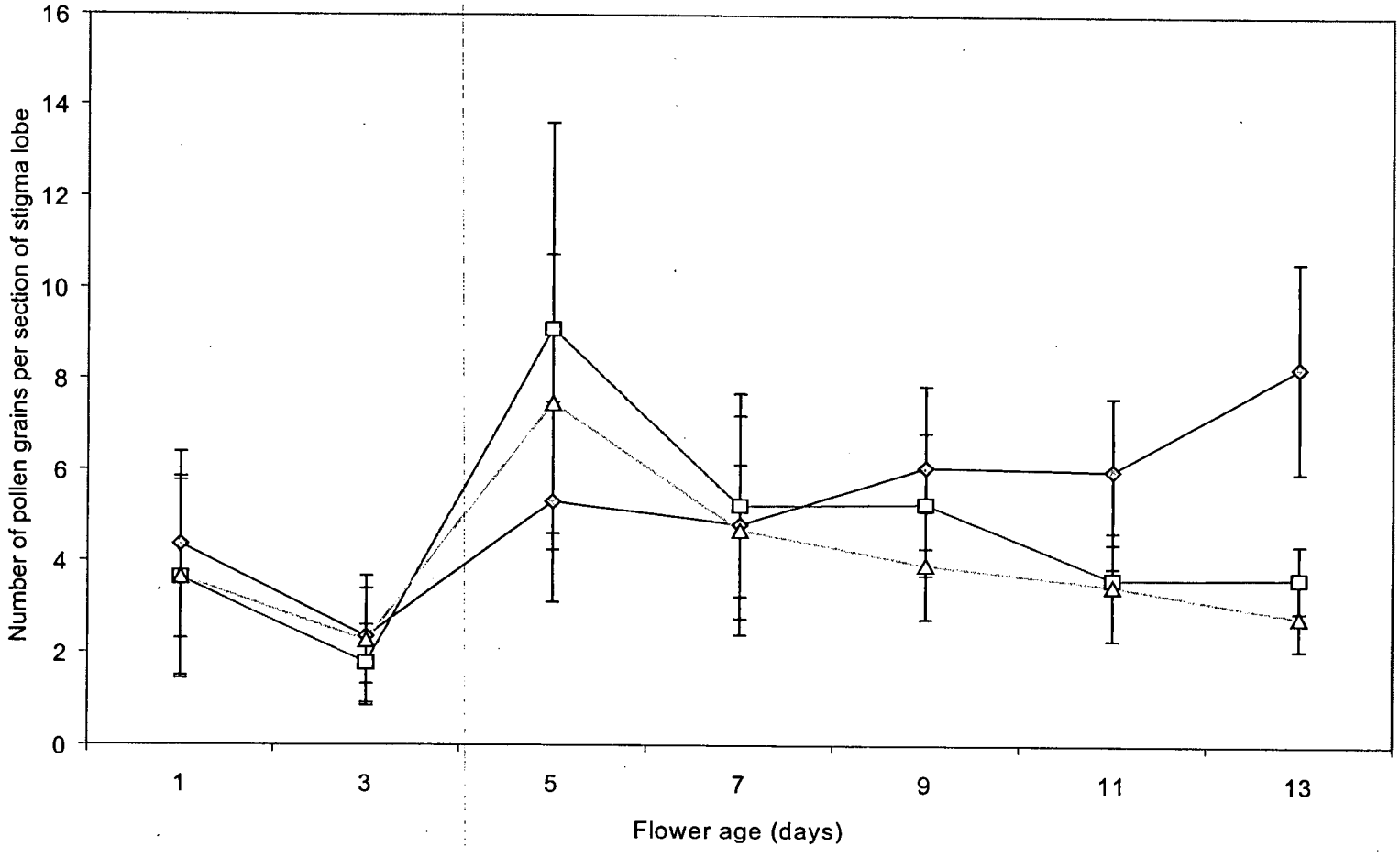

Fig. 2.3. Number of pollen grains deposited on stigma lobes for the first (diamonds), second (squares) and third (triangles) 448-μm-sections of stigma lobes. n=10-12 flowers for all points. Error bars are standard errors.

load of self pollen is substantially greater than the pollen loads adhering to stigmas of un-bagged flowers of a similar age (Fig. 2.3). Because flowers were both bagged (to protect them from visitors) and sprayed with insecticide (to kill any squatter insects resident inside flowers), these loads of self pollen are presumed to represent autogamous deposition through mechanical dislodgment of pollen from anthers onto stigmas of the same flower.

In contrast, flowers which had been emasculated and then bagged (plus insecticide) for seven days carried very small numbers of pollen grains (0.87±0.20 grains per stigma, n=10).

Fruit and seed set: effect of pollinator exclusions

Fruit set

Of the 1140 flower tags put out in January 1999, 1042 (91.4%) were located 12 months later when fruits were harvested. There was a significant effect of treatment ($F_{5,55}$=64.4, P<0.001) and tree ($F_{11,55}$=2.5, P<0.025) on fruit set (Fig. 2.4). Fruit set for both un-bagged/intact and un-bagged/emasculated flowers were similar (Bonferroni test P>0.9) and exceeded 80% (Fig. 2.4). Fruit set for the bagged/intact treatments with insecticide (32.0±7.7%) and without insecticide (37.6±4.1%) were also comparable (Bonferroni test P>0.9). Fruit set for the bagged/emasculated flowers without insecticide (13.6±2.3%) in which insect squatters were still present was significantly lower than in both bagged/intact treatments (Bonferroni tests P<0.001 and 0.05<P<0.1 for treatments 3 and 4, respectively). Fruit set for bagged/emasculated flowers with insecticide was very low (5.6±1.9%) although not significantly different to fruit set for bagged/emasculated flowers without insecticide (Bonferroni test P>0.9). While the occasional fruit set in bagged emasculated flowers with insecticide could be the result of wind-borne pollen, stigmas of flowers which were emasculated and bagged with insecticide treatment carried very small pollen loads (<1 grain per stigma). The low fruit set in treatment 6 is therefore presumed to be the result of occasional incomplete emasculation of flowers and this treatment was not included in the subsequent analyses.

I pooled treatments 1 and 2 (un-bagged flowers) and treatments 3 and 4 (bagged/intact flowers) and retained treatment 5 (bagged/emasculated flowers, no insecticide), and tested for an effect of treatment ($F_{2,22}$=132.8, P<0.001) and tree ($F_{11,33}$=2.2, 0.05<P<0.1) on fruit set. Fruit set in un-bagged flowers (84.0±3.7%) was significantly greater than in bagged/intact flowers (34.0±4.4%) (Bonferroni test P<0.001), while fruit set in bagged/intact flowers

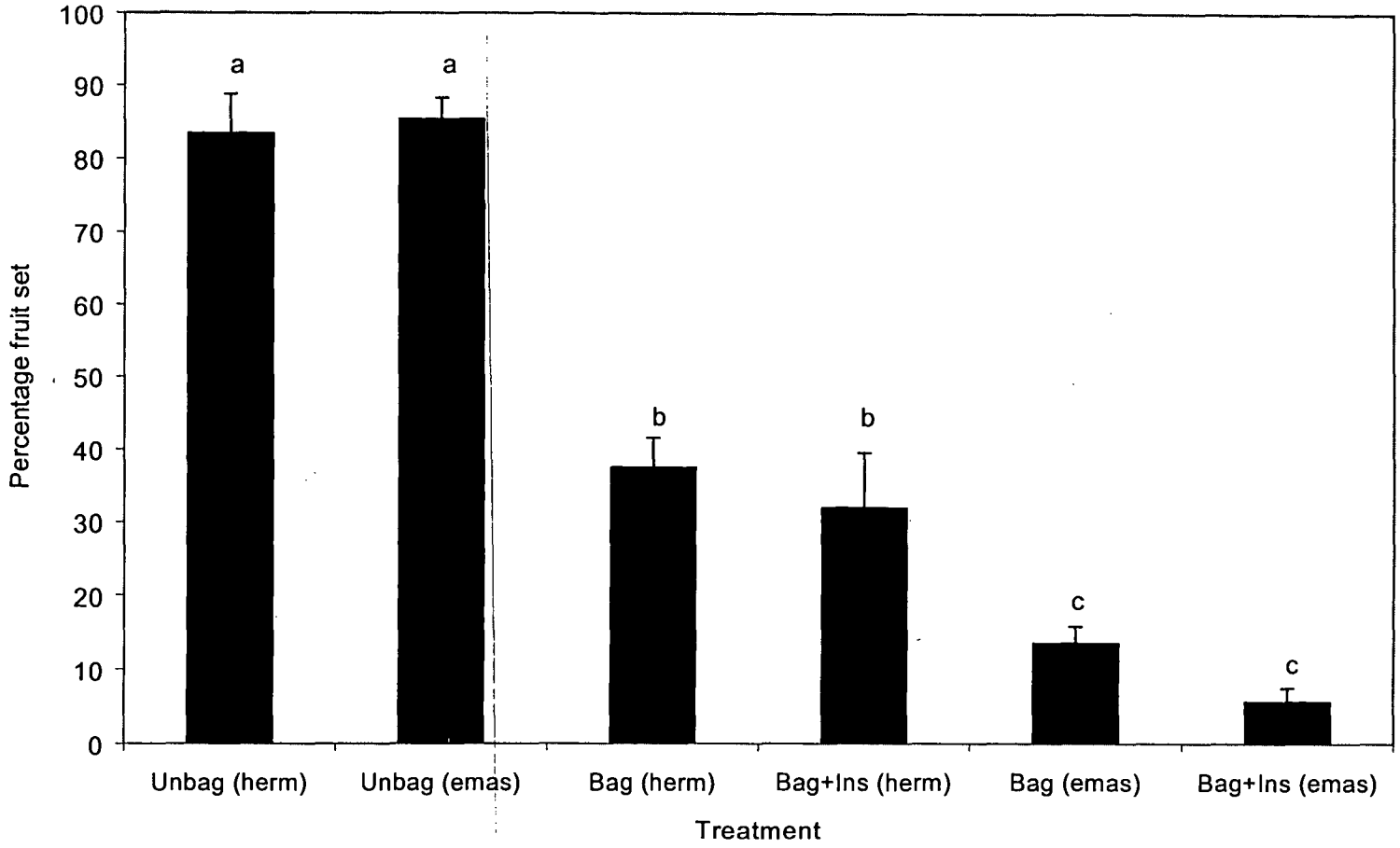

Fig. 2.4. Percentage fruit set of unbagged/hermaphrodite flowers, unbagged/emasculated flowers, bagged/hermaphrodite and bagged/emasculated flowers without insecticide, and bagged/hermaphrodite and bagged/emasculated flowers with insecticide treatment. n=12 trees for all treatments. Bars with the same letter were not significantly different (Bonferroni test $P<0.05$). Error bars are standard errors.

was significantly greater than in bagged/emasculated flowers without insecticide (13.6±2.3%) (Bonferroni test $P<0.001$),

Fruit Opening

All fruits of a single tree failed to open, and this tree was excluded from the analysis. There was a significant effect of treatment ($F_{4,36}=14.9$, $P<0.001$) and tree ($F_{10,46}=4.88$, $P<0.001$) on fruit dehiscence (Fig. 2.5). As for fruit set, I pooled treatments 1 and 2 (un-bagged flowers) and treatments 3 and 4 (bagged/intact) and tested for an effect of treatment ($F_{2,18}=18.7$, $P<0.001$) and tree ($F_{10,28}=3.3$, $P<0.025$) on fruit dehiscence. Fruit dehiscence for un-bagged flowers (80.4±7.0%) was significantly greater than for bagged/intact flowers (51.0±11.0%) (Bonferroni test $P<0.05$), while fruit dehisecence in bagged/intact flowers exceeded that of bagged/emasculated flowers (23.11±9.8%) (Bonferroni test $P<0.01$).

Seed Set

There was a significant effect of treatment ($F_{4,243}=27.7$, $P<0.001$) and tree ($F_{10,243}=14.2$, $P<0.001$) on seed set (Fig. 2.6). I pooled treatments 1 and 2 (un-bagged flowers) and treatments 3 and 4 (bagged/intact flowers) and tested for an effect of treatment and tree on seed set ($F_{2,245}=53.8$, $P<0.001$ and $F_{10,245}=15.0$, $P<0.001$, respectively). Seed set in un-bagged flowers (36.3±1.4%) was significantly greater than in both bagged/intact (16.3±1.6%) and bagged/emasculated treatments (14.3±3.3%) (Bonferronu tests $P<0.001$) which did not differ significantly (Bonferronu test $P>0.5$).

Fruit Weights

Fruits for three trees were accidentally discarded before weighing, so the following analysis includes data from only eight trees. Fruit weights closely followed the pattern of seed set (Fig. 2.7), with a significant effect of treatment ($F_{4,267}=54.73$, $P<0.001$) and tree ($F_{7,267}=31.69$, $P<0.001$) on fruit weights. I pooled treatments 1 and 2 (un-bagged flowers) and treatments 3 and 4 (bagged/intact flowers) and tested for an effect of treatment and tree on fruit weight ($F_{2,269}=109.95$, $P<0.001$ and $F_{7,269}=32.20$, $P<0.001$, respectively). Mean fruit weight in un-bagged flowers (0.116±0.003 g) was significantly greater than in both bagged/intact (0.076±0.003 g) and bagged/emasculated treatments (0.062±0.007 g) (Bonferroni tests $P<0.001$) which did not differ significantly (Bonferroni test $P>0.4$).

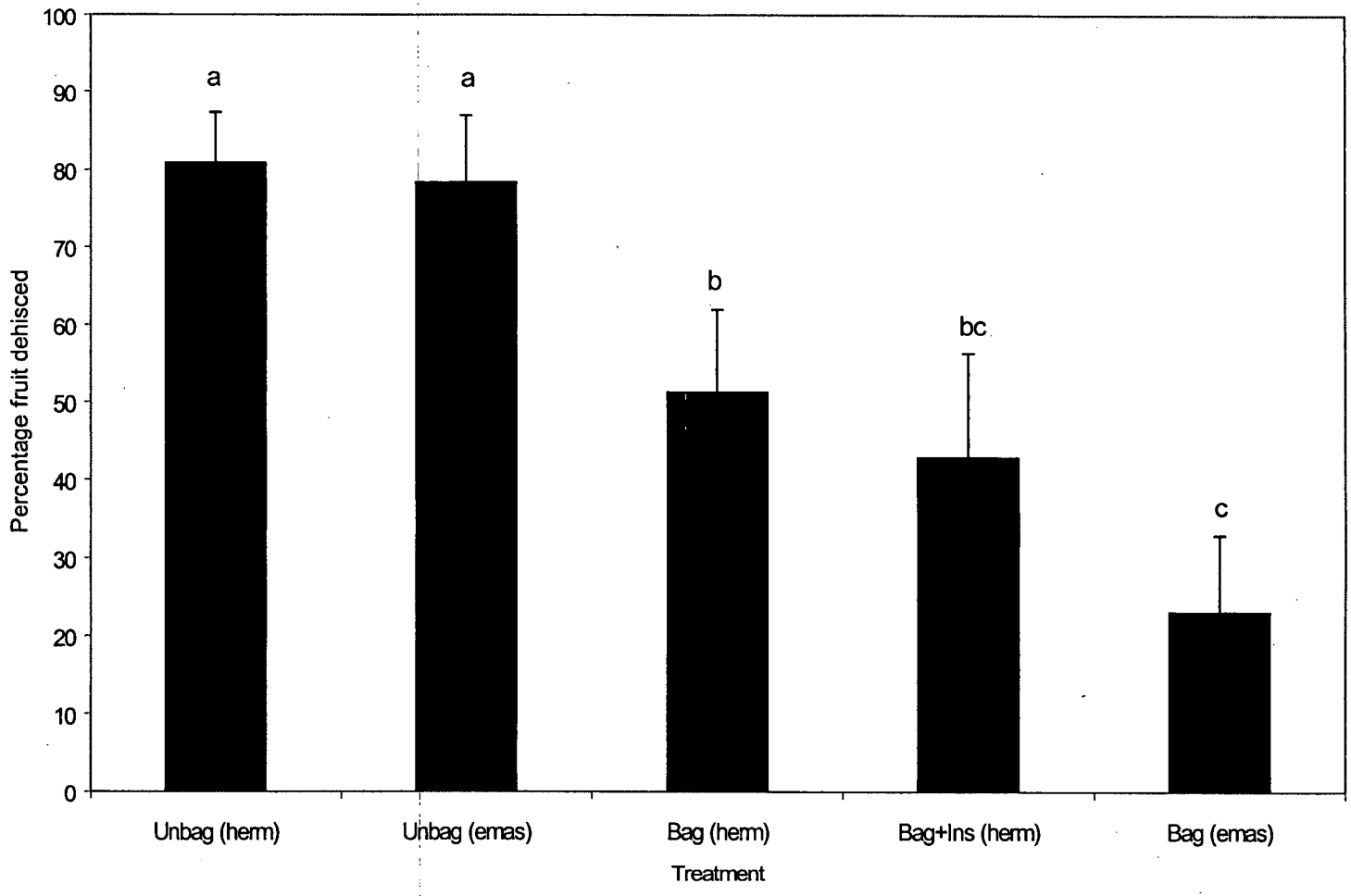

Fig. 2.5. Percentage of fruit which dehisced for unbagged/hermaphrodite flowers, unbagged/emasculated flowers, bagged/hermaphrodite and bagged/emasculated flowers without insecticide, and bagged/hermaphrodite flowers with insecticide treatment. n=9-11 trees for all treatments. Bars with the same letter were not significantly different (Bonferroni test $P<0.05$). Error bars are standard errors.

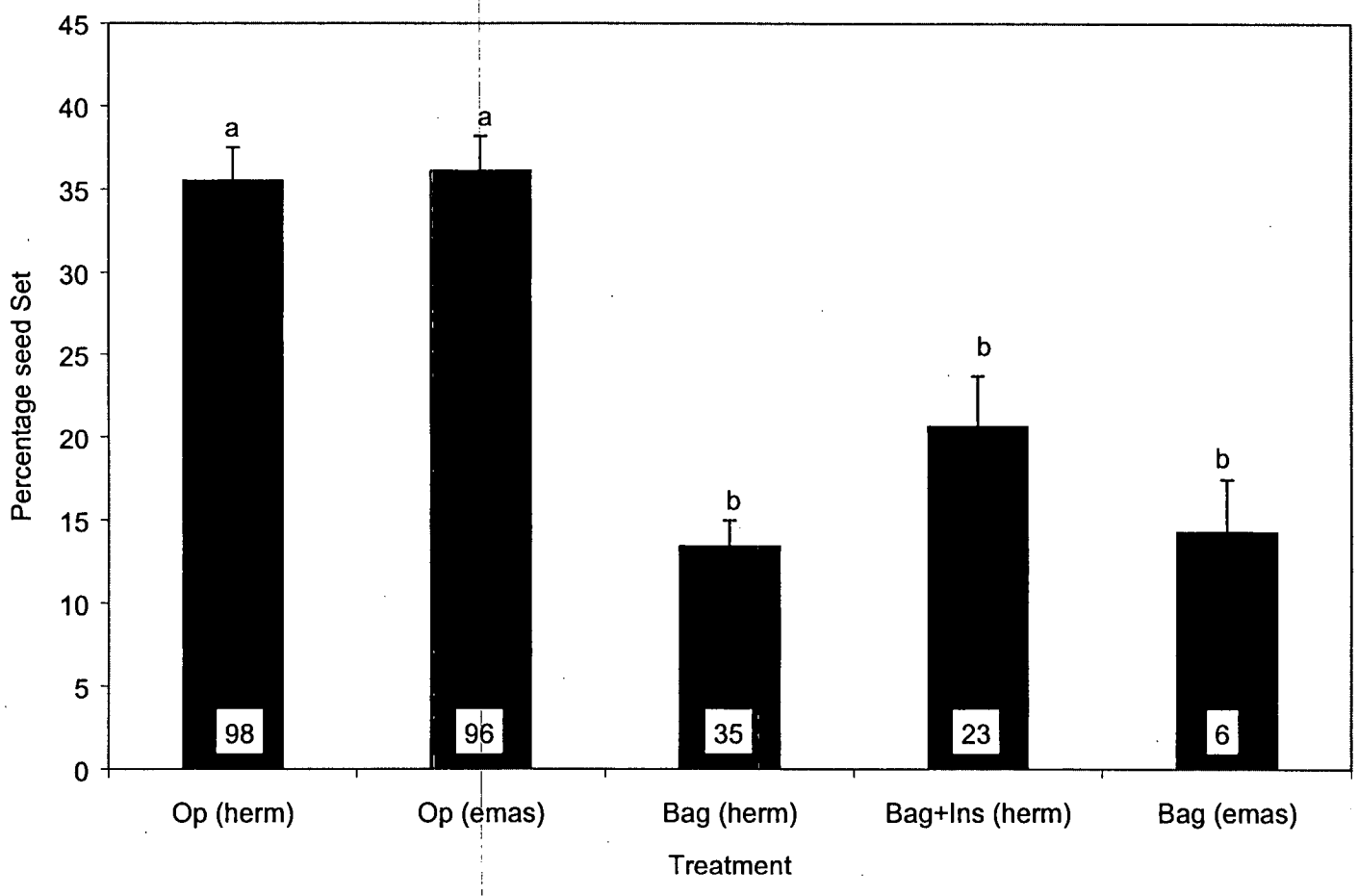

Fig. 2.6. Percentage seed set for unbagged/hermaphrodite flowers, unbagged/emasculated flowers, bagged/hermaphrodite and bagged/emasculated flowers without insecticide, and bagged/hermaphrodite flowers with insecticide treatment. Sample sizes are given at the bottom of bars. Bars with the same letter were not significantly different (Bonferroni test $P<0.05$). Error bars are standard errors.

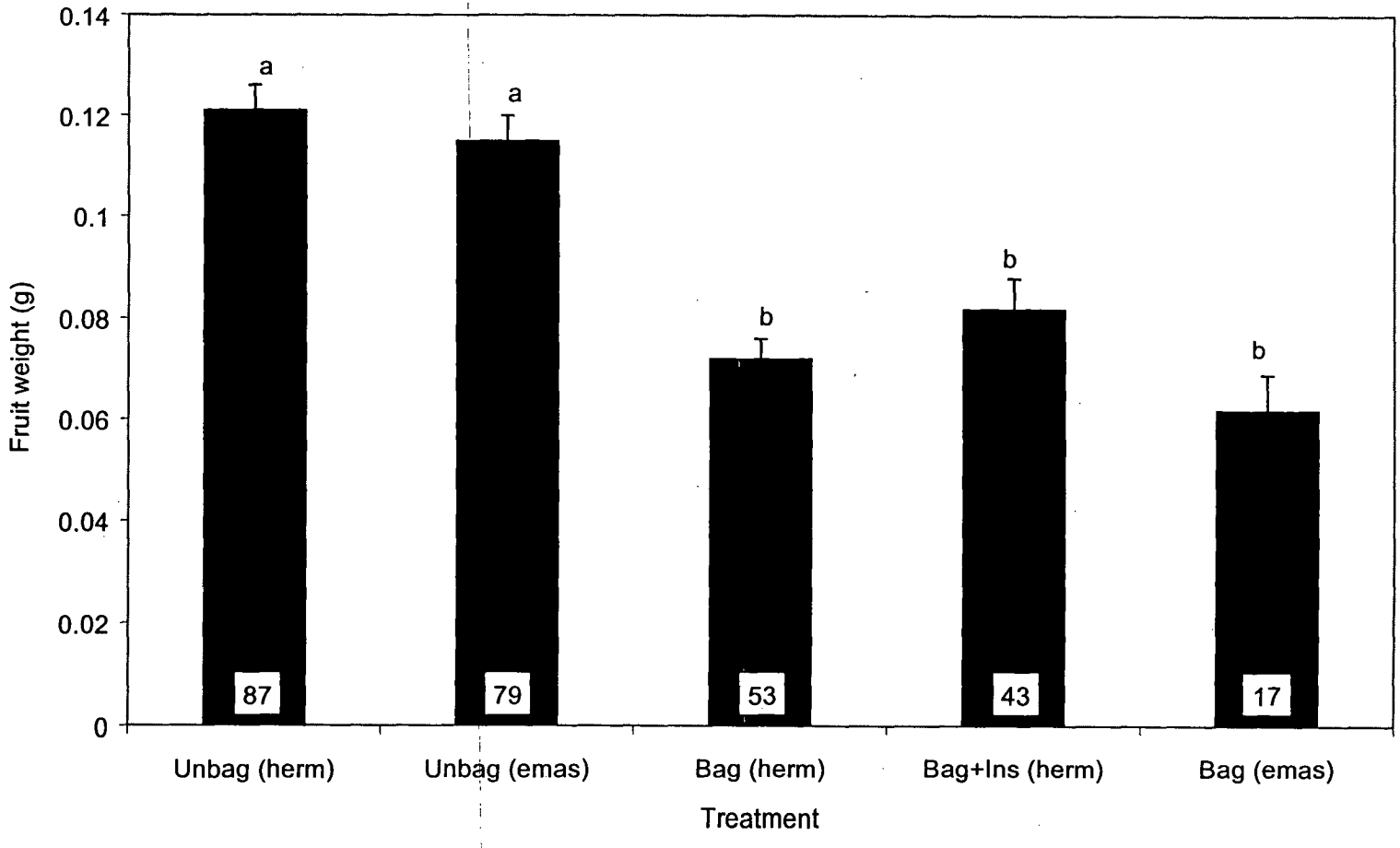

Fig. 2.7. Mean fruit weight for unbagged/hermaphrodite flowers, unbagged/emasculated flowers, bagged/hermaphrodite and bagged/emasculated flowers without insecticide, and bagged/hermaphrodite flowers with insecticide treatment. Sample sizes are given at the bottom of bars. Bars with the same letter were not significantly different (Bonferroni test $P<0.05$). Error bars are standard errors.

Seed set and fruit weight

There was a strong correlation between percentage seed set and fruit weight ($F_{1,172}$=187.1, r^2=0.54, P<0.001, n=174) with the linear regression line described by the equation Fruit Set = 0.0014(Fruit Weight) + 0.0696.

Discussion

Results from the present study suggest that flowers of *E. lucida* are protandrous, as suggested by Ettershank and Ettershank (1992), with an initial male phase lasting approximately 6-7 days, and stigma receptivity commencing around day 7. Dehiscing of anthers was staggered over the initial 4-5 days of anthesis, and the released pollen was steadily removed by native insects (mainly flies and beetles) and feral honeybees. In un-bagged flowers, the numerous insect visits (over 20 visits/flower/day; see Chapter 3) ensured that most of the pollen had been removed from flowers by the commencement of stigma receptivity. In contrast, in bagged flowers (visitors excluded), pollen rapidly accumulated in flowers. Some mechanical dislodgment of pollen presumably occurred in bagged flowers as the proportion of anthers bearing pollen did not approach 100%. However, up to 60% of anthers carried pollen at the commencement of the female phase, and bagged flowers still contained substantial quantities of pollen at the end of their life (Fig. 2.2).

The separation of stigma lobes and anthers in female flowers varied between trees, with the position of stigma lobes ranging from several mm below to several mm above the level of the anthers. However, in all flowers there was some overlap between the anthers and the receptive surface of the stigma lobes, resulting in substantial potential for autogamous deposition of self pollen (*cf.* Eckert and Schaefer 1998; Kalisz *et al.* 1999). Furthermore, in bagged flowers in which pollen was retained throughout the female phase, stigmas carried heavy loads of self pollen, with the number of self-pollen grains deposited over the entire female phase exceeding the number of ovules per ovary (mean number of ovules/ovary=43.42±0.68, n=262).

Flowers of *E. lucida* were partially self fertile. Bagged intact flowers with a super-abundance of self pollen on stigmas set low but appreciable quantities of fruit (34%) and seed (16%). Given the super-abundance of self pollen on the stigmas of bagged flowers, this relatively low level of seed-set suggests substantial abortion of selfed seed, either through deleterious/lethal recessives (Charlesworth and Charlesworth 1987; Wiens *et al.* 1987), or a late-acting self-incompatibility mechanism (Seavey and Bawa 1986; Waser and Price 1991; Ramsey *et al.* 1993; Ramsey and Vaughton 2000).

In un-bagged flowers, stigmas received substantial pollen loads throughout anthesis, with an upper estimate of 1700 pollen grains deposited on stigmas during the female phase. This number of grains is substantially greater than the mean number of ovules per ovary and suggests that, at least in the present study, seed set in *E. lucida* is not limited by the quantity of pollen deposited in flowers. However, counts of the number of pollen grains fails to take into account the quality of pollen, most notably whether pollen is cross or self (Silander and Primack 1978; Motten 1983; Ramsey *et al.* 1993). Where self-pollination (either autogamous or geitonogamous) is substantial, large pollen loads on stigmas may still be accompanied by low fruit and/or seed set through the usurping of ovules by self pollen in both self-incompatible species (Silander and Primack 1978; Waser and Price 1991; Juenger and Bergelson 2000), and in self-compatible species with early-acting inbreeding depression (Ramsey *et al.* 1993; Ramsey 1995; Ramsey and Vaughton 2000).

Despite a superabundance of pollen on stigmas, seed set in un-bagged *E. lucida* flowers was relatively low (36%) and highly variable (range 3-85%). This suggests that natural seed set in *E. lucida* results from a combination of geitonogamous self and cross pollination, with substantial abortion of the selfed ovules. Autogamous selfing was unlikely in un-bagged flowers as the majority of pollen had been removed by the onset of stigma receptivity (Fig. 2.2). It is possible that pollen deposited autogamously onto unreceptive stigmas during the male phase remained in place and viable until stigmas became receptive (*cf.* Ramsey and Vaughton 2000). However, the absence of any difference in fruit or seed set between un-bagged/intact and un-bagged/emasculated flowers (Fig. 2.4 and 3.6) indicates that this form of ovule usurping did not occur in *E. lucida* flowers.

Significant levels of geitonogamous self pollination appear highly likely in *E. lucida* given the simultaneous presentation of large numbers (up to several thousands) of male and female flowers on individual trees. Both native-insect visitors and the introduced honeybee typically visit multiple flowers per tree on any one approach, and do not appear to discriminate between flower sex (see Chapter 3). Pollen deposited during an insect's visit will therefore include a mixture of geitonogamous and cross pollen, with the relative proportions of cross and self pollen deposited in a flower a (highly variable) function of visitor type (Mitchell and Waser 1992), length of visit (Thomson and Plowright 1980; Mitchell and Waser 1992; Klinkhamer and de Jong 1993), the extent of pollen carryover (Thomson and Plowright 1980; Price and Waser 1982; Thomson 1986; Hessing 1988; Robertson 1992), and the position of the flower in the

visitation sequence (Thomson and Plowright 1980; Price and Waser 1982; Rademaket *et al.* 1999).

Further work is now required to determine the precise proportions of self and cross pollen deposited by different pollinators, and the levels of selfing that result.

Squatter insects and selfing in E. lucida

The principal squatter insects in *E. lucida* flowers were small beetles, particularly staphylinids from the subfamily Aleocharinae, thrips and spiders. Mites, hemipterans and collembola were also occasionally found in flowers. Evidence from the bagging plus insecticide treatments suggests these squatters may effect some self pollination in *E. lucida* flowers.

Self pollination by squatters may be autogamous (i.e. the 'facilitated' selfing of Lloyd and Schoen [1992], in which pollen is transferred by a vector from anthers to stigma within a flower), or geitonogamous through movement of the insects among nearby flowers on the same branch. Facilitated selfing by squatter insects is likely to have been very pronounced in bagged flowers in the female phase in which the anthers carried abundant pollen. However, fruit set for bagged/intact flowers with insecticide (i.e. without squatters; 32.0±7.7%) and without insecticide (i.e. with squatters; 37.6±4.1%) were not significantly different (Fig. 2.4). Presumably in bagged intact flowers with abundant self pollen, high levels of autonomous selfing (i.e. passive or non-vector mediated deposition of pollen from anthers to stigma within a flower) swamped any facilitated contribution by squatters so that the removal of squatter insects did not affect fruit set. Fruit set in bagged/emasculated flowers without insecticide was appreciable however (13.6±2.3%), and is presumably the result of squatter-mediated geitonogamous selfing within the bagged branch. Baker and Cruden (1991) also found that thrip and aphid squatters were responsible for a significant proportion of the fruit set of *Ranunculus sceleratus* and *Potentilla rivalis*, although they did not distinguish between autonomous and geitonogamous modes of pollen transfer.

Curiously, although the fruit set of bagged emasculated *E. lucida* flowers (geitonogamously selfed via squatters) was less than in bagged and intact flowers (autonomously selfed), the seed set for the two treatments were similar (14.3±3.3% and 16.3±1.6%, respectively). Presumably, not all *E. lucida* flowers on a bagged branch supported squatter insects. Those flowers without squatters would not have received pollen and failed to set fruit, leading to a reduced mean fruit set in bagged and emasculated flowers). However those bagged and emasculated flowers which were utilised by squatters apparently received

substantial amounts of geitonogamous self pollen as the squatter insects moved around the flower, and set a similar number of seeds as bagged and intact flowers which received a super-abundance of self.

High fruit set and low seed set in E. lucida

Flowers of *E. lucida* which were open to pollinators set large numbers of fruit (>80% mean fruit set) with relatively low mean seed set (36%) and a highly variable numbers of seed set per fruit (range 3.1 - 84.9% of ovules matured). The production of 'surplus' flowers (ie. <100% fruit set) and the selective abortion of fruits is extremely common in plants (Sutherland and Delph 1984; Sutherland 1986), and a number of hypotheses have been put forward to explain the phenomenon (reviewed by Stephenson 1981). The production of 'excess' ovules (ie. <100% sed set) has received considerably less attention (Stephenson 1981; Sutherland and Delph 1984). Lack of attention to the phenomenon of selective ovule abortion may be a reflection of the modular gamete packaging practiced by plants, where ovules are typically matured or aborted as packages or fruits (Burd 1994). One outcome of such modular packaging of seeds is that, under most conditions, a plant should maximise its seed production by maturing only those fruits in which the majority of ovules have been successfully fertilised, rather than by maturing many (still expensive) fruits containing few seeds (Lloyd 1980; Stephenson 1981; Burd 1994).

The high fruit set and low seed set of *E. lucida* goes against this expectation, and suggests that *E. lucida* trees may be wasting energy in allowing many fruits with few seeds to develop. However, in mass-blooming, self-compatible species with inbreeding depression, most flowers on a plant will receive substantial and highly variable quantities of geitonogamous self pollen, leading to a low and highly variable seed set as a result of the usurping of a proportion of ovules by self pollen. Under these circumstances, a plant may effectively maximise its total seed output by maturing many fruits with few seeds, rather than relying on (an unpredictable) supply of fruits in which all or a majority of seeds have been successfully outcrossed. Such a pattern appears to occur in *E. lucida*, and may be a common form of seed packaging in mass-flowering, self-compatible plants which are subject to high levels of geitonogamous selfing.

Chapter 3. Honeybees versus native anthophiles: natural and exotic pollinators of *E. lucida*

Abstract

I used video cameras to record diurnal and nocturnal insect visitors to flowers of *E. lucida*. Flowers received visits from a broad range of native diurnal insects (dipterans;16 families, coleopterans; 6 families, hymenopterans; 5 families, and lepidopterans; 2 families) and nocturnal insects (tipulid flies, elaterid beetles, blattellid cockroaches, and geometrid and pyralid moths), as well as from the introduced honeybee. *E. lucida* flowers also supported a range of squatter insects (mainly thrips, staphylinid beetles, and spiders) which used the flowers as a semi-permanent refuge. Total visitation rates (honeybees plus native insects) varied substantially, ranging from <2 to >25 visits per flower per 10-hour day. Visitation rates by honeybees were increased by the presence of commercial hives. Visitation rates by native insects also varied substantially between sites, years and over a season (range 1.0-22.0 visits per flower per 10-hour day), but did not appear to be consistently related to the presence or absence of commercial hives. Nocturnal visitation rates were <2 visits per flower per 10-hour night. Large dipterans (≥5 mm) and to a lesser extent large coleopterans (≥3 mm) were the most important native pollinators of *E. lucida*. Honeybees visited on average <10 flowers per tree during a visitation sequence, returned with around 5-7 mg sugar per bee, and were estimated to have visited between two and four trees during a foraging bout. Honeybees appear to be efficient pollinators of *E. lucida* flowers.

Introduction

Insect pollinated plants often receive visits from a wide range of taxa from the order level down (Herrara 1987; Inouye and Pyke 1988; Ollerton 1996; Waser *et al.* 1996; Hingston and Potts 1998). Such diverse assemblages of visitors may also vary in composition from place to place, over a season, and between years (Herrara 1987; Ashman and Stanton 1991; Ollerton 1996; Fishbein and Venable 1996). At a crude level, the most frequent visitors to flowers are often assumed to be the most important pollinators of a plant. However, pollinator effectiveness is a product of a number of separate components in addition to visitation frequency, including visit duration and the specific behaviour of the insect within the flower (Schemske and Horvitz 1984; Herrara 1988; Mitchell and Waser 1992; Klinkhamer and de Jong 1993). Very subtle aspects of an insect's behaviour can influence its effectiveness as a pollinator, including the number of flowers visited per plant (Klinkhamer and de Jong 1993; Hodges

1995), the pattern of visits to male and female flowers (Motten *et al.* 1981; Freitas and Paxton 1998), and the quality of pollen transferred (i.e. whether it is cross or self; Motten *et al.* 1981; Price and Waser 1982; de Jong *et al.* 1993). Furthermore, pollinators typically pick up as well as deposit pollen, and pollinator effectiveness may also be a function of the relative amounts of pollen removed against the amount deposited in flowers (e.g. Wilson and Thomson 1991; Gross and MacKay 1998).

The relative effectiveness with which an insect pollinates a flower may be often measured directly as seed set in response to a defined pollination event (usually a single visit) (e.g. Motten *et al.* 1981). The relative importance of insect visitors as pollinators of a plant can also be measured indirectly using visitation rates combined with other aspects of vector behaviour, including pollen loads, behaviour within flowers, and the likelihood that these behaviours will lead to pollen transfer (Spears 1983). While indirect measures do not directly measure a flower's reproductive success following visitation, they can be quickly collected in the field, allowing an investigation of variation in the composition and relative efficiency of different pollinator assemblages under a diversity of plant and pollinator conditions.

The breeding system and pollinators of *E. lucida* are poorly known. A single study by Ettershank and Ettershank (1992; also Ettershank 1993) recorded a very broad range of invertebrates visiting *E. lucida* flowers covering eight orders of insects and two arachnid orders. Most of these species were nectar and pollen feeders and were considered to be potential pollinators, while the remainder were predators and parasites (Ettershank and Ettershank 1992). The most frequent native insect visitors were tabanid leatherwood flies (*Scaptia* spp.), while feral and hive honeybees were also abundant at flowers. Although Ettershank and Ettershank (1992) did not consider the potential effectiveness of honeybees as pollinators of *E. lucida*, they suggested the honeybees were unlikely to be depressing the levels of native insect pollinators.

This study investigated the anthophilous fauna of *E. lucida* flowers over three summers from 1998 to 2000. I used video cameras to record diurnal and nocturnal insect visitors and their behaviour at flowers. Native insects were grouped into broad visitor guilds at the order level (large/small Diptera, large/small Coleoptera, native Hymenoptera, and Lepidoptera). Information was obtained on the composition of the diurnal fauna and rates of visitation to *E. lucida* flowers from fourteen sites around Tasmania. I also obtained data on the behaviour of insects at flowers and their relative pollen loads, and the number of flowers visited per tree during a visitation sequence. I used the data on diurnal visitation rates, relative pollen loads and the likelihood of vector-stigma contact

during visits to calculate the relative importance of different pollinator groups between sites and years and over a season. I also sampled the 'squatter' insect fauna inside *E. lucida* flowers. In addition, I obtained more detailed data on honeybee foraging patterns at *E. lucida* flowers, including the amount of nectar sugar removed per flower visit, nectar loads of bees returning to the hive, and the influence of nectar levels on visit duration and the number of visits per tree.

Methods

Study sites

I recorded insect visits to *E. lucida* flowers at fourteen sites from four locations around Tasmania. See *Study Sites* section for details.

Video data: diurnal insect visitors

I used two Sony Handycam Video 8 cameras (model nos. CCD-TR501E: 15x optical zoom and CCD-TR511E: 18x optical zoom) mounted on camera tripods (height=1.5 m) to record diurnal insect visitors to flowers. All video data were gathered on warm clear days with a temperature maximum of >18°C. Video data were gathered at three times of day (between 0900-1100 hours, 1200-1400 hours and 1500-1700 hours; hereafter the 1000-, 1300- and 1600-hour sampling sessions) on 3-5 days for each site. For the Waratah and Queenstown sites, video data were gathered either by two people working simultaneously, one at an apiary and one at a non-apiary site, or by a single person working at an apiary site for one hour and then moving to a non-apiary site for the next hour of a sampling session. The order of the apiary/non-apiary sites was alternated on different days. For the three Link Road sites, one person worked at one site while another person gathered data from the two other sites during each two-hour sampling session. The order in which the Link Road sites were visited was alternated on different days. I collected data 'simultaneously' from apiary and non-apiary sites on the same days in order to reduce the effect of variation in insect activity between days, allowing a more robust comparison of insect visitation rates between sites with and without commercial apiaries (see Chapter 8).

For each sampling session at each site, a camera was placed 2-3 m from a flowering tree, trained on a set of 4-10 *E. lucida* flowers and run for a 10-minute segment, after which the camera was moved to a new set of flowers on the same tree for a second 10-minute segment. This was continued for 4-5 segments over approximately one hour. Data from a single sampling session usually came from a single tree. Different trees were used on the three sampling sessions during a single day, and where possible different sets of trees were used on different days

at a site. Data were recorded on Sony 8 mm (90 minute) cassettes run on long-play mode (i.e. 180 minutes playing time per tape).

Tapes were later analyzed using a video recorder and TV monitor. For each 10-minute segment, the number of flowers in clear view on the monitor was assessed, and only insect visits to these flowers recorded. For each 10-minute segment, I scored all floral visits, with visitors recorded as honeybees, large (≥ 5 mm) or small diptera (<5 mm), large coleoptera (≥ 3 mm), native Hymenoptera, or Lepidoptera. Honeybees were further divided into those with a golden-coloured abdomen (typical of Itanlianate-race hive bees) and those which were dull-coloured or black (typical of feral bees; Ettershank and Ettershank 1992). Small Coleoptera (<3 mm) were not scored as they tended to remain in flowers for longer than the 10-minute segment. Wherever possible, I also classified insect visitors down to the lowest taxonomic level possible. I also noted the behaviour of insect visitors at flowers, including whether the visit was an approach (contact with flower <1 second) or a feeding visit (flower contact >1 second with obvious feeding behaviour), the duration of each feeding visit (to the nearest second), and whether the insect collected nectar or pollen or both. Where possible, I also recorded whether the insect appeared to make clear contact with the flower stigma during a visit. I calculated visitation rates as the number of visits per flower per 10-minute video segment. I then estimated the average number of visits a flower would be likely to receive over a 10-hour day by calculating the mean visitation rate for the 1000-, 1300- and 1600-hour sampling sessions combined, and multiplying this figure by 6 (=visits per hour) and by 10 (= visits per 10-hour day).

Video data: nocturnal insect visitors

I used the nightshot function of the CCD-TR511E video camera to record insect visits to *E. lucida* flowers on two warm, clear nights at MAY in early February. At 2200 hours (approximately one hour after dusk) the camera was trained on a flowering branch (10-15 flowers) and left running for a full 3-hour tape. Tapes were retrieved on the morning of the following day. Different trees were used on different nights.

Squatter insects in E. lucida *flowers*

Squatter insects were sampled from a flower by holding a plastic container (mouth diameter=50 mm) under a flower and snipping the pedicel with scissors so that the flower fell into the container together with any small insects present within. I collected twenty samples of flowers (n=30 flowers per sample) from the Waratah sites at various times of day in February 1998. Flower samples

were stored in 70% alcohol. Samples were sorted by first gently shaking the container to dislodge insects still inside flowers, then removing the flowers and placing the alcohol together with any insects into a petri dish. For thrip larvae, I counted the number of larvae for three random fields of view using a binocular microscope (6x magnification), and used the mean count per field of view to estimate total number of thrips in the entire petri dish. All other insect groups were scored as total counts.

Insect Pollen Loads

I investigated the pollen loads carried by the most common insect visitors to *E. lucida* flowers: honeybees, large and small flies, large and small beetles and native bees. Individual insects were captured while feeding on or in the near vicinity of *E. lucida* flowers and placed in an insect killing jar (Australian Entomological Supplies, NSW) with a dose of pyrethrum-based insecticide. A small (*ca.* 2 mm^3) cube of agar on the end of a toothpick was dabbed 3-4 times on the ventral surface of the insect (thorax and abdomen) to pick up pollen grains. The agar cube was then placed on a microscope slide with a drop of lactophenol-aniline blue stain, left to stand for several minutes and then sealed under a coverslip using clear nail-polish. For each pollen slide, I obtained a count of the number of pollen grains for 10 random fields of view (250x magnification) under a light microscope, and then calculated the mean number of pollen grains per field of view for each slide.

Nectar sugar removed per visit by honeybees

I examined the percentage of available nectar sugar removed during a single visit by a honeybee. Data were collected from four different trees over several warm days from various apiary sites in January and February 1999. Preceding an observation period, I measured the nectar sugar per flower in a sample of 10-15 flowers from a tree by picking flowers, washing out nectar sugar with two rinses of 20 μL distilled water and measuring the sugar concentration using a hand-held refractometer (see Appendix 1 for details of washing method). The tree was then watched for 10-13 nectar-feeding visits by honeybees. The duration (in seconds) of each visit was recorded, and after the honeybee had vacated the flower the flower was immediately picked and the remaining nectar sugar measured as above. The mean nectar sugar per flower preceding the observation period was taken as the average 'background' level of nectar sugar on the tree, and the amount extracted by honeybees calculated as this background level minus the weight of nectar sugar remaining in a flower after a honeybee visit.

Nectar sugar levels, visit duration and the number of flowers visited by honeybees

I examined the effect of the mean weight of nectar sugar per flower on the duration of honeybee visits for 11 different trees. In addition to the four trees described in the previous section, I observed honeybee visits to another seven trees at various apiary sites over several warm days in February 2000. I measured the weight of nectar sugar in a sample of 8-15 flowers from each tree to obtain an estimate of background sugar per flower. I then recorded the duration (in seconds) of 20-30 honeybee visits by 5-9 individual honeybees per tree, For these seven trees, I also attempted to follow individual honeybees as they foraged to record the number of flowers visited per visitation sequence on a tree. For these data, I used only those honeybees for which I was able to accurately monitor arrival to and departure from the tree, and where I was able to follow the honeybee throughout its visitation sequence.

Total flowers on a tree and the number of flowers visited by honeybees

The influence of the total number of flowers on a tree on the number of flowers visited by honeybees during a visitation sequence was investigated in February 2000 at MAY. I chose four trees ranging from small (<50 flowers) to moderately sized (>1000 flowers) and obtained an estimate of the total number of flowers on each tree. For the three smaller trees, I counted the total number of flowers three times, and took the average of these three counts (mean n=32, 93 and 290 flowers). For the larger tree, I estimated the total number of flowers by approximating the shape of the flowering crown (a cone), and estimating its basal radius and height. I then estimated the number of flowers per 30 cm^2 at 10 points over the crown, and used the average of these counts to estimate the number of flowers per square metre. Combining the estimate of flowering-crown area with flower density gave an estimate of the total flowers on the tree (n=1053 flowers). Over several warm days I observed honeybees foraging on these four trees, and recorded the total number of flowers visited per visitation sequence for those bees where I was able to accurately monitor arrival to and departure from the tree, and where I was able to follow the honeybee throughout its visitation sequence.

Nectar loads of honeybees and floral nectar-levels over a day

The volume and concentration of nectar and total weight of nectar sugar carried by honeybees returning to their hives were examined at MAY over two warm days in February 2000. At four times over the day (0900, 1200, 1500 and 1700

hours), I captured 10-21 honeybees returning to their hives by carefully pinning the bee as it landed at the hive entrance. I used only bees without obvious pollen sacs on their corbiculae (i.e. those returning after a nectar-collecting fight). The captured bee was gently squeezed between the thumb and forefinger to expel its nectar load, the droplet of nectar drawn up into a 20 μL micropipette, and nectar volume measured from the height of the column. The nectar was then expelled onto the prism of a hand-held refractometer (Universal Type model no. 505-I: 0-90% sugar wt/wt). Concentration readings in sugar wt/wt were converted to sugar wt/vol before calculating sugar weights. I also measured the volume and concentration of nectar and total weight of nectar sugar in a sample of flowers at 0900, 1200, 1500 and 1700 hours on the same days. I used 5 μL micropipettes to probe around the base of the stamens to pick up and measure the volume of liquid nectar in flower. Because of the low volume and viscosity of the floral nectar and the requirement of the refractometers for at least 4 μL for a reading (see Appendix 1), I used the same micropipette to extract nectar from a series of flowers (n=1-6) until the required volume had been gathered, after which a refractometer reading was taken. Concentrations were therefore usually an average using the combined nectar from several flowers.

Results

Composition of fauna

Overall, I recorded 1193 10-minute video segments (199 hours total footage), and observed a total of 7737 flowers, giving a total of 1289.5 'flower-hours' of observation. During this time I observed a total of 1402 flower visits (including occasional repeat visits by the same insect), 552 (39.4%) of which were by honeybees and 850 (60.6%) by native insects. *E. lucida* flowers received visits from a wide range of native insects from five orders and a total of 34 families (Table 3.1). The most diverse diurnal visitors were dipterans (16 families) and beetles (6 families), while a range of native wasps, native bees and butterflies also visited flowers during the day. The range of native bees visiting flowers was probably underestimated as most bees were very small and difficult to identify from the videos. *E. lucida* flowers received occasional visits during the night, primarily from tipulid flies and moths (Table 3.1), although nocturnal visitation rates were substantially lower than daytime rates (see below). *E. lucida* flowers also supported a range of squatter insects using the flowers as a semi-permanent refuge. Thrips (mean±se=12.8±1.2/field of view, or 175.4 thrips per flower), beetles (mean±se=6.2±1.0/flower) and spiders

Table 3.1. Native insects visiting *E. lucida* flowers. * nocturnal visitor.

Diptera		
	Tipulidae*	
	Mycetophylidae	
	Bibionidae	
	Chloropidae	
	Lauxaniidae	
	Sepsidae	
	Syrphidae	
		Melangyna spp.
		Eristalinae
	Conopidae	
	Heleomyzidae	
	Platystomatidae	
	Stratiomyidae	
	Empididae	
	Pelecorhynchidae	
	Tabanidae	
		Scaptia spp.
	Muscidae	
	Calliphoridae	
	Tachinidae	
		Rutilia spp
		Senostoma spp
Coleoptera		
	Elateridae*	
	Staphylinidae	
		Aleocharinae
	Mordellidae	
		Mordella sp.
	Cantharidae	
		Chauliognathus sp.
		Chauliognathus nobilitatus
		Chauliognathus lugubris
	Cerambycidae	
	Lycidae	
		Metriorynchus sp.
	Oedomeridae	
		Ischnomera lineata
		Copidita sp.
Hymenoptera		
	Sphecidae	
	Ichneumonidae	
	Gasteruptionidae	
	Colletidae	
		Callomelitta picta
		Euryglossa sp.
	Formicidae	
		Camponotus consobrinus
Blattodea		

Blattellidae*	
Lepidoptera	
Geometridae*	
Pyralidae*	
Papilionidae	
	Graphium macleayanum
Hesperiidae	
	Hesperilla sp.

(mean±se=1.3±0.2/flower) were the most common squatters, while ants, colembola, mites and lepidopteran larvae were occasionally found in flowers.

Honeybees were observed visiting *E. lucida* flowers at all sites and in all years, and made up between 8.3 and 75.7% of the total visits to flowers (Table 3.2). For all sites, years and seasons combined, honeybees made up a significantly greater proportion of total flower visits when commercial apiaries were present (51.6% of total) compared to where commercial apiaries were absent (23.0% of total; X^2=139.6, P<0.001, df=3). The vast majority of bees (98.0%) near apiaries were 'golden' coloured and were presumed to be hive bees. In contrast, at non-apiary sites the majority of bees (80.5%) were dull-coloured or black and were presumed to be bees from feral swarms (*cf.* Ettershank and Ettershank 1992; Sudgen and Pyke 1991).

Large dipterans (≥ 5 mm) tended to be the principal native visitor to flowers, ranging from 8-100% of total native visits (Table 3.2). Large dipterans were the predominant native visitor at all sites and times except at LR1 in 1998 when hymenopterans were the predominant visitors, and at MAY in early and mid season when coleopterans predominated (Table 3.2). Small dipterans (<5 mm), small coleopterans (<3 mm) and hymenopterans (mainly native bees) made up a variable proportion of total visits at different sites and times, while butterflies were only occasionally recorded visiting flowers (Table 3.2).

Visitation rates

I expressed the video data as the number of visits per flower for each 10-minute segment. I then calculated the mean visitation rate for the various visitor taxa (data for days and times of day were combined), and multiplied this mean value by 60 to give an estimate of the total number of visits a flower would receive over a 10-hour day (Table 3.3). Overall, the estimated total daily visitation rate (honeybees plus natives) to *E. lucida* flowers varied by greater than an order of magnitude, ranging from <2 to >25 visits per flower per 10-hour day (Table 3.3). Visitation rates by native insects also varied widely between sites, ranging from 1.0-22.2 native visitors per flower per day, with large dipterans the most frequent native visitor to flowers at the majority of sites (Table 3.3).

Visitation rates by honeybees tended to be greater near commercial apiaries compared to non-apiary sites, although visitation rates by honeybees at some apiary sites (e.g. WAR3 and QT1) was surprisingly low given the number of hives nearby (Table 3.3). The effect of introducing commercial hives on honeybee visitation rates was readily apparent at the Link Road where honeybee visits increased by a factor of 3.6 and 26.3, respectively, after the introduction of 100 hives to LR1 and LR2 in 2000 (Table 3.3). In contrast, at the control site

Table 3.2. The composition of the insect fauna visiting flowers of *E. lucida* at fourteen sites from four locations: Waratah (3 apiary and 3 control sites); Queenstown (2 apiary and 2 control sites); the Link Road (3 sites in1998: no hives, and in 2000: hives at LR1 and LR2); and at Maydena in early (no hives), mid (34 hives) and late season (102 hives).
Shown are the number of hives at site, the number of 10-minute video segments, total flowers observed, total number of insect visitors, total number of honeybees and native insects (% of total visitors in brackets), and the composition of the native insect fauna (% of total native insects in brackets).

							Native Visitors (% of total natives in brackets)				
Location/Site	Hives	Seg.	Fl.	Total	Hbs (% total)	Total Nats (% total)	Dipt. (lrge)	Dipt. (smll)	Coleopt.	Hym.	Lep.
Waratah											
WAR1	50	67	500	181	107 (59.1)	74 (40.9)	56 (75.7)	11 (14.9)	4 (5.4)	3 (4.1)	-
WAR2	60	63	423	156	49 (31.4)	107 (68.6)	66 (61.7)	30 (28.0)	10 (9.4)	1 (0.9)	-
WAR3	80	45	387	41	19 (46.3)	22 (53.7)	16 (72.7)	2 (9.1)	1 (4.6)	3 (13.6)	-
WAR4	-	61	377	140	34 (24.3)	106 (75.7)	72 (67.9)	12 (11.3)	14 (13.2)	8 (7.6)	-
WAR5	-	64	390	151	25 (16.6)	126 (83.4)	105 (83.3)	16 (12.7)	3 (2.4)	2 (1.6)	-
WAR6	-	46	377	19	9 (47.4)	10 (52.6)	10 (100.0)	-	-	-	-
Queenstown											
QT1	120	91	516	61	24 (39.3)	37 (60.7)	19 (51.4)	8 (21.6)	5 (13.5)	5 (13.5)	-
QT2	100	57	289	54	30 (55.6)	24 (44.4)	19 (75.0)	-	4 (16.7)	2 (8.3)	-
QT3	-	81	500	14	6 (42.9)	8 (57.1)	5 (62.5)	1 (12.5)	2 (25.0)	-	-
QT4	-	46	291	17	5 (29.4)	12 (70.6)	4 (33.3)	2 (16.7)	3 (25.0)	3 (25.0)	-
Link Road											
LR1.1998	-	75	436	47	15 (31.9)	32 (68.1)	13 (40.6)	-	4 (12.5)	15 (46.9)	-
LR1.2000	100	36	245	37	28 (75.7)	9 (24.3)	6 (66.7)	2 (22.2)	1 (11.1)	-	-
LR2.1998	-	75	423	24	2 (8.3)	22 (91.7)	10 (45.5)	1 (4.6)	2 (9.1)	9 (40.9)	-
LR2.2000	100	42	313	60	31 (51.7)	29 (48.3)	22 (75.9)	-	-	7 (24.1)	-
LR3.1998	-	74	400	66	11 (16.7)	55 (83.3)	40 (72.7)	2 (3.6)	5 (9.1)	7 (12.7)	1(1.8)
LR3.2000	-	39	250	23	8 (34.8)	15 (65.2)	8 (53.3)	1 (6.7)	1 (6.7)	3 (20.0)	2 (13.3)
Maydena											
Early	-	60	293	98	23 (23.5)	75 (76.5)	23 (30.7)	-	35 (46.7)	16 (21.3)	1 (1.3)
Mid	34	93	710	87	37 (42.5)	50 (57.5)	4 (8.0)	-	28 (56.0)	18 (36.0)	-
Late	102	78	617	126	89 (70.6)	37 (29.4)	20 (54.1)	2 (5.4)	-	15 (40.5)	-

Table 3.3. Estimated number of visits per flower per 10-hour day by honeybees and native insects to *E. lucida* flowers at fourteen sites from four locations: Waratah (3 apiary and 3 control sites); Queenstown (2 apiary and 2 control sites); the Link Road (3 sites in1998: no hives, and in 2000: hives at LR1 and LR2); and at Maydena in early (no hives), mid (34 hives) and late season (102 hives).

Location/Site	No. hives	Total	Honeybees	Natives	Dipt. (lrge)	Dipt. (smll)	Coleopt.	Hymen.	Lepidopt.
Waratah									
WAR1	50	21.8	12.4	9.4	7.2	1.1	0.5	0.6	-
WAR2	60	25.1	8.0	17.1	10.1	5.4	1.4	0.2	-
WAR3	80	7.0	3.3	3.7	2.9	0.2	0.1	0.5	-
WAR4	-	21.7	4.6	18.8	12.0	2.1	2.8	1.9	-
WAR5	-	23.2	3.2	22.2	18.7	2.5	0.5	0.5	-
WAR6	-	2.7	1.2	1.5	1.5	-	-	-	-
Queenstown									
QT1	120	6.8	2.4	4.4	2.3	1.0	0.5	0.6	-
QT2	100	11.9	7.3	4.6	3.4	-	0.7	0.5	-
QT3	-	1.6	0.6	1.0	0.6	0.1	0.3	-	-
QT4	-	4.1	0.9	3.2	0.9	0.7	0.7	0.9	-
Link Road									
LR1.1998	-	7.1	2.1	5.0	1.8	-	0.6	2.6	-
LR1.2000	100	9.4	7.5	1.9	1.1	0.5	0.3	-	-
LR2.1998	-	3.2	0.3	2.9	1.4	-	0.3	1.2	-
LR2.2000	100	14.3	7.9	6.4	4.5	-	-	1.9	-
LR3.1998	-	9.3	1.6	7.7	5.7	-	0.7	1.3	-
LR3.2000	-	6.6	2.9	3.7	2.4	0.1	0.2	0.6	0.4
Maydena									
Early	-	18.9	4.5	14.4	4.6	-	6.3	3.4	0.1
Mid	34	7.9	3.4	4.5	0.3	-	2.5	1.7	-
Late	102	13.1	9.4	3.7	2.0	0.3	-	1.4	

(LR3) honeybee visits increased by a factor of only 1.8 between years (Table 3.3). Similarly at Maydena, the visitation rate by honeybees tended to increase as more hives were introduced to the site (Table 3.3).

There was substantial variation in the visitation rate by native insects between years (Link Road) as well as over a season (Maydena). At the Link Road, the number of native insect visits per flower per day declined by 62.0% at LR1 but increased by 120.7% at LR2 after the introduction of 100 hives, while at LR3 the visitation rate by native insects declined by 52.0% in the second year (Table 3.3). There was also substantial variation in the number of native insect visits per flower per day over a season at MAY, with native visitation rates tending to decline over the season as more hives were introduced (Table 3.3). In general however, visitation rates by native insects did not appear to be consistently related to the presence or absence of commercial bees (Table 3.3; see also Chapter 8 for more discussion of the impacts of hive honeybees on native visitors to *E. lucida*).

Visitation rates at night were extremely low. I recorded only seven visits (one tipulid fly, one cockroach and four moths) to 15 flowers on the 3-hour tape on the first night, and three visits (all by tipulid flies) to 9 flowers on the second night at MAY. This gave an estimated visitation rate of 1.6 and 1.1 visits per flower per 10-hour night for the two nights, respectively.

Insect behaviour, visit duration, pollen loads and contact with stigmas

Honeybees took both nectar and pollen from *E. lucida* flowers. Pollen collecting bees engaged in obvious raking behaviour as they straddled the anthers and gathered pollen onto their body. Nectar collecting bees repeatedly probed for nectar, either from the side of the flower around the base of the stamens or through the anthers from above. Occasionally a bee appeared to both probe for nectar and rake pollen during a single visit, although most bees appeared to be gathering only nectar or only pollen. Honeybees (nectar and pollen collecting bees combined) spent an average of 9.6±0.4 seconds in a flower visit (data for all sites combined), although visit duration depended on the nectar content of the flower (see below). Honeybees carried the greatest pollen loads of all insect visitors (mean±se=11.5±2.5 grains/field of view, n=7). Over 99% of grains belonged to *E. lucida*, with only an occasional grain from a myrtaceous species (either *Eucalyptus* or *Leptospermum*) recorded from honeybees. On those visits (n=215) where the contact of the honeybee within the flower could be adequately viewed, the bee appeared to make at least one contact with the flower stigma on 86.1% of cases. Contact with the stigma occurred principally between the ventral surface of the honeybee's thorax and abdomen, occasionally

with the legs and less frequently with the head as the bee probed for nectar from above. Honeybees often alighted on a flower, made a rapid probe but did not feed (visit duration <1 second), and appeared to be making a rapid assessment of flower suitability. Honeybees also often approached flowers (to within several mm) without making contact and then appeared to reject the flower, indicating the bees were also able to assess flowers from a short distance away.

The most common large dipterans were syrphids, tabanids, calliphorids, tachinids and the larger muscid flies. Most syrphid flies were observed taking pollen although some syrphids also took nectar, while the other flies were nectar feeders. The feeding behaviour of large nectar-feeding dipterans was similar to honeybees, with the fly either alighting on the anthers and probing for nectar from above or collecting nectar from the side of the flower around the base of stamens. The latter behaviour was more common in the smaller flies in this category and was less likely to involve contact with the centrally located stigma. The largest flies (e.g. pelecorhynchids, body length >20 mm) may also have had less stigma contact as their straddled feeding-position tended to raise the body of the fly above the level of the anthers and stigma. Stigma contact was most likely in intermediate sized dipterans (body length around 10-12 mm), such as *Rutilia* spp. and *Scaptia* spp., as the fly moved about on the surface of the massed anthers and probed for nectar. Overall, large dipterans spent an average of 28.8±2.1 seconds during a flower visit (data for all sites combined). Large dipterans carried moderate loads of pollen on their ventral surface (3.5±1.6 grains/field of view, n=12). The majority of pollen grains (>99%) belonged to *E. lucida*, with only an occasional *Anodopetatum biglandulosum* grain recorded. Large dipterans appeared to make at least one contact with the flower stigma on 58.4% of cases (n=154). Contact with the stigma occurred principally between the ventral surface of the fly's thorax and abdomen, as well as with the legs and less frequently with the head as the fly probed for nectar from above. Large dipterans also frequently investigated a flower without going on to feed, although such behaviour appeared less systematic than was the case for honeybees.

Small dipterans (<5 mm) almost invariably accessed nectar from the side of the flower around the base of the stamens (presumably because their mouth-parts were too short to probe from above), and seldom made contact with the flower stigma (contact on 29.7% of cases, n=37). I was unable to record feed-time for small dipterans as their small size and continual movement in and around the stamens made it difficult to continually monitor their presence and feeding activity in flowers. However, small dipterans appeared to spend relatively long in flowers, with some visits lasting throughout the 10-minute

video segment. Smal dipterans carried relatively small loads of *E. lucida* pollen on their ventral surface (1.22±0.44 grains/field of view, n=9).

Small coleopterans (families Staphylinidae and Oedomeridae) were difficult to continuously monitor in flowers, but were observed taking pollen (by crawling up the filaments and feeding at the anthers) and nectar from around the base of the stamens. Small coleopterans carried very little pollen on their ventral surface (0.04±0.03 grains/field of view, n=9). Large coleopterans varied in their feeding behaviour. Large cantharids often walked over flowers without feeding but may have deposited pollen onto stigmas while doing so. Where feeding was observed, cantharids took pollen in a clumsy mess-and-spoil fashion typical of large beetles in flowers. Mordellid and lycid beetles fed by landing abruptly on the massed anthers and feeding on pollen. Large coleopterans carried moderate loads of pollen on their ventral surface (3.99±1.33 grains/field of view, n=21). The majority of beetles carried only *E. lucida*, while some beetles also carried pollen from a myrtaceous species (either *Eucalyptus* or *Leptospermum*). Overall, large coleopterans contacted the flower stigma on 85.0% of cases (n=20).

Native hymenopterans included both native wasps (families Sphecidae, Ichneumonidae and Gasteruptionidae), native bees (family Colletidae) and occasional ants. Wasps were observed taking nectar from around the base of the stamens and only occasionally by probing from above. Native bees typically took both nectar and pollen during a single visit. Native bees frequently moved over the anthers as they worked a flower, and stigma contacts were observed in 63.6% of cases (n=11). The mean duration of visits by native bees and wasps was 45.6±7.0 seconds. Native wasps and bees carried relatively small loads of pollen on their ventral surface (1.08±0.05 grains/field of view, n=6). Ants were only occasionally seen feeding at flowers, although ants were often seen moving about on the stems and leaves. Ants fed from around the base of the stamens and seldom moved into the vicinity of the flower stigma.

Butterflies were only occasionally recorded at *E. lucida* flowers from the video footage, although *Graphium macleayanum* was otherwise observed to be quite common at some sites on certain days. *G. macleayanum* visits were of short duration (mean visit length 4.1±0.7 seconds, n=19; non-video observations), with the butterfly feeding on nectar by lightly resting on the massed stamens and probing through the anthers to the nectaries at the base of the flower.

Relative importance of different pollinators

I calculated a relative 'pollination score' for each pollinator type except lepidopterans for the different sites, years and seasons. Scores were calculated as: visitation rate*percentage stigma contacts per visit*pollen loads. I then ranked pollinators in order of relative importance (Table 3.4). In almost all cases, honeybees were the most important pollinator of *E. lucida* flowers. The only exceptions were LR2.1998, where honeybees and large dipterans had comparable scores, and WAR5 where large dipterans were slightly more important than honeybees (Table 3.4). Large dipterans were the second most important pollinator at most sites, except at Maydena where coleopterans were more important in early and mid season (Table 3.4). Hymenopterans and small dipterans appear to play a minor role as pollinators *of E. lucida* (Table 3.4). The introduction of hives to the two apiary sites at the Link Road in 2000 was not accompanied by any major shift in the rankings of pollinators due to the presence of feral bees in the control year (Table 3.4).

Nectar levels, per visit nectar consumption by honeybees, and visit duration and sequence

The background levels (mean±se) of nectar sugar for Trees 1, 2, 3 and 4 were 3.20±0.51, 0.52±0.11, 0.31±0.07 and 0.23±0.05 mg per flower, while the mean weight of nectar sugar remaining in flowers after a honeybee visit was 0.99±0.26, 0.37±0.11, 0.15±0.04 and 0.08±0.03 mg per flower, respectively. This represented a per visit mean consumption of 2.21, 0.14, 0.16 and 0.15 mg, or 69.1%, 26.9%, 51.6% and 65.2% of the available sugar, respectively.

There was a significant correlation between the background levels of nectar sugar on a tree and the mean duration of honeybee visits, with feed time tending to increase with the amount of sugar per flower (linear regression $F_{1,9}=16.71$, $r^2=0.65$, $P<0.01$; Fig. 3.1). However, there was no relationship between mean nectar sugar per flower and the number of flowers visited during a visitation sequence (linear regression $F_{1,4}=0.14$, $r^2=0.03$, $P>0.5$), or the total number of flowers on a tree and the number of flowers visited during a visitation sequence (linear regression $F_{1,2}=2.47$, $r^2=0.55$, $P>0.2$). The mean number of flowers visited by honeybees during a visitation sequence was 9.14±1.71 flowers per tree (range=2.4-12.8, n=7).

I also attempted to follow native insects throughout a visitation sequence. However, this proved impossible for the smaller insects (small dipterans, small coleopterans and native bees), while the larger coleopterans remained too long in individual flowers for it to be practicable to pursue them. Large dipterans were also difficult to follow as they moved quickly and eratically between

Table. 3.4. Relative importance of different pollinators to flowers of *E. lucida*.

Location/Site	Relative importance
Waratah	
WAR1	Hbs > Ldip > Col > Sdip = Hym
WAR2	Hbs > Ldip > Col > Sdip > Hym
WAR3	Hbs > Ldip > Col > Hym > Sdip
WAR4	Hbs > Ldip > Col > Sdip > Hym
WAR5	Ldip > Hbs > Col > Sdip > Hym
WAR6	Hbs > Ldip
Queenstown	
QT1	Hbs > Ldip > Col > Sdip = Hym
QT2	Hbs > Ldip > Col > Hym
QT3	Hbs > Ldip = Col > Sdip
QT4	Hbs > Ldip > Col > Hym > Sdip
Link Road	
LR1.1998	Hbs > Ldip > Col > Hym
LR1.2000	Hbs > Ldip > Col > Sdip
LR2.1998	Hbs = Ldip > Col > Hym
LR2.2000	Hbs > Ldip > Hym
LR3.1998	Hbs > Ldip > Col > Hym
LR3.2000	Hbs > Ldip > Col > Hym > Sdip
Maydena	
Early	Hbs > Col > Ldip > Hym
Mid	Hbs > Col > Hym > Ldip
Late	Hbs > Ldip > Hym > Sdip

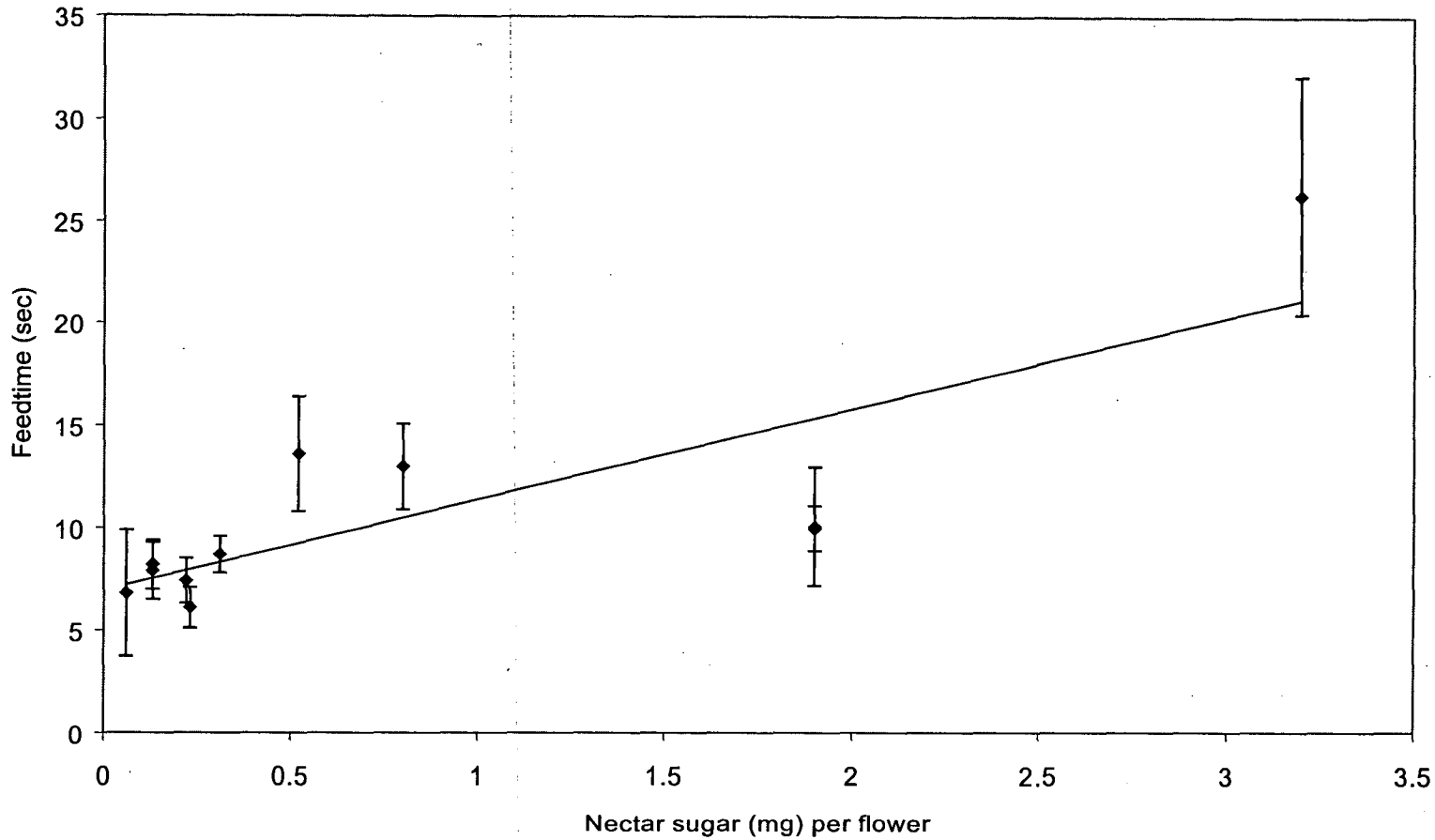

Fig. 3.1. Relationship between mean nectar sugar per *E. lucida* flower for eleven trees and mean duration of honeybee visits to flowers. Linear regression line is also shown. Error bars are standard errors.

flowers. However I managed to follow a sample (n=11) of large dipterans from a range of trees and sites, with a mean of 4.27±0.94 flowers per tree visited during a visitation sequence. I also followed a number of butterflies (all *G. macleayanum)*, which visited 5.09±1.00 flowers per tree (n=23).

Floral nectar and nectar loads of honeybees

Flowers contained small volumes (<4 μL) of a relatively dilute nectar (27.68±2.27% wt/vol) at 0900 hours (Fig. 3.2). Nectar volumes declined to <1 μL per flower by mid-afternoon as concentrations increased to >70% wt/vol, after which volumes increased and nectar concentration decreased slightly as ambient temperatures cooled in the late afternoon (Fig. 3.2). The concentration of nectar carried by honeybees returning to the hive closely followed the concentration of floral nectar (Fig. 3.3). Nectar volumes carried by honeybees ranged from 9.5-11.9 μL and also tended to follow the general pattern of nectar volume in flowers (Fig. 3.3). Combining the values of nectar volume and concentration for each bee gave an estimate of the weights of nectar sugar carried by returning honeybees (see Table 3.5). Sugar loads remained remarkably consistent over the day, ranging from 5.5-7.1 mg of sugar per bee. The mean weight of sugar carried by a returning honeybee for all bees combined was 6.81±1.23 mg sugar per bee.

I used the data on the weight of sugar available per flower over a day (Fig. 3.2), the percentage of sugar removed by a bee during a visit (mean for the four trees described above was 53.2% of background sugar removed per visit, rounded off to 50%), and the mean weight of sugar carried by a returning bee over the day to calculate the number of visited flowers required to make up the returning load (Table 3.5). The mean weight of sugar available per flower decreased during the warmest part of the day, then increased again towards late afternoon (Table 3.5). The number of flowers required to make up the returning load followed a similar pattern, ranging from 12.3 flowers in the morning to 31.5 flowers in mid-afternoon (Table 3.5). The number of trees which would have to be visited to make up this number of flowers (assuming 9 flowers visited per tree, see above) ranged from 1.4 (i.e. two trees) in the morning to 3.5 (i.e. four trees) by mid-afternoon (Table 3.5).

Discussion

Native pollinators of E. lucida

Flowers of *E. lucida* received visits from a broad range of insects encompassing 5 orders and 34 families. Dipterans were both the most diverse group (16

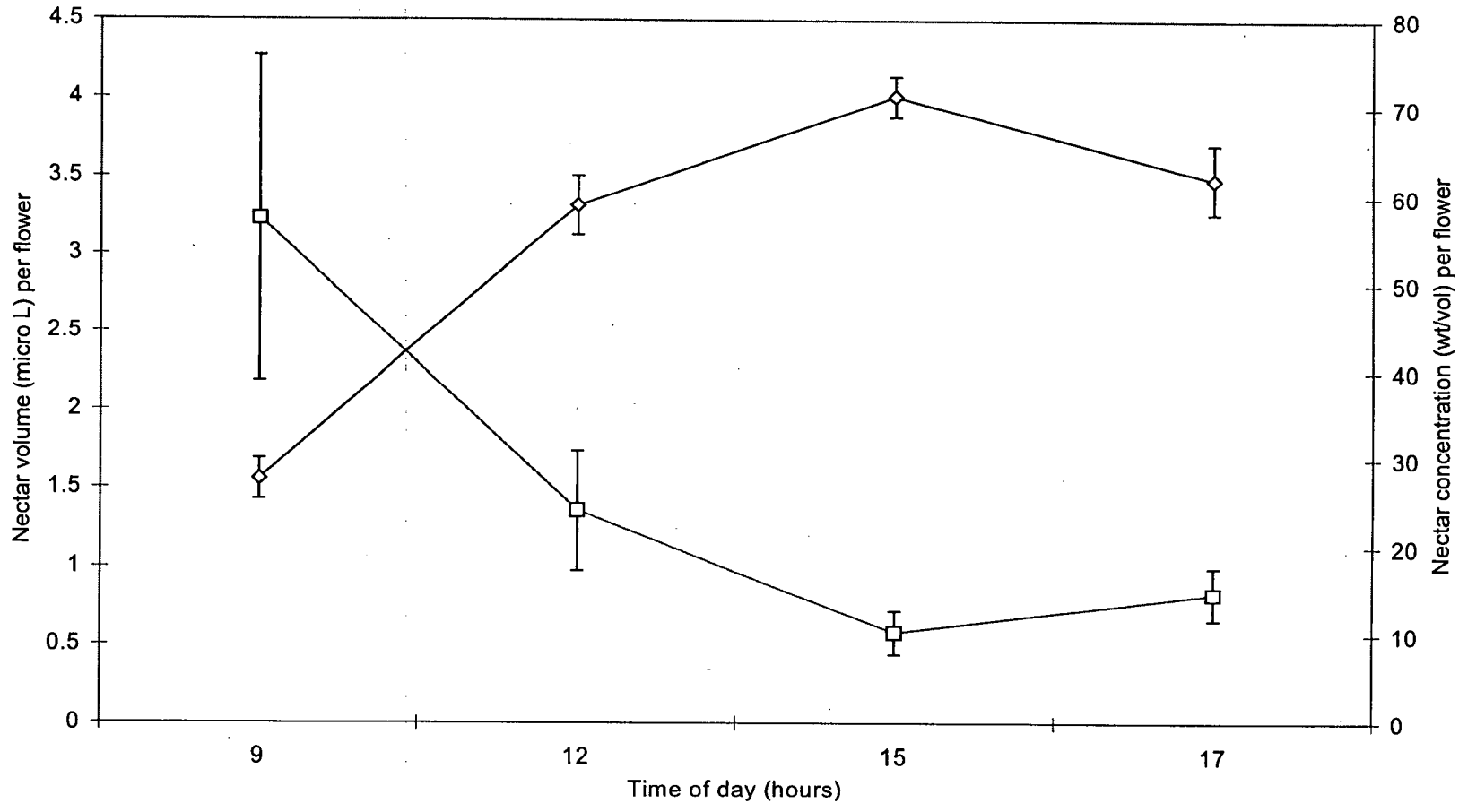

Fig. 3.2. Volume (squares) and concentration (diamonds) of nectar in *E. lucida* flowers over the day. n=10-15 for all points. Error bars are standard errors.

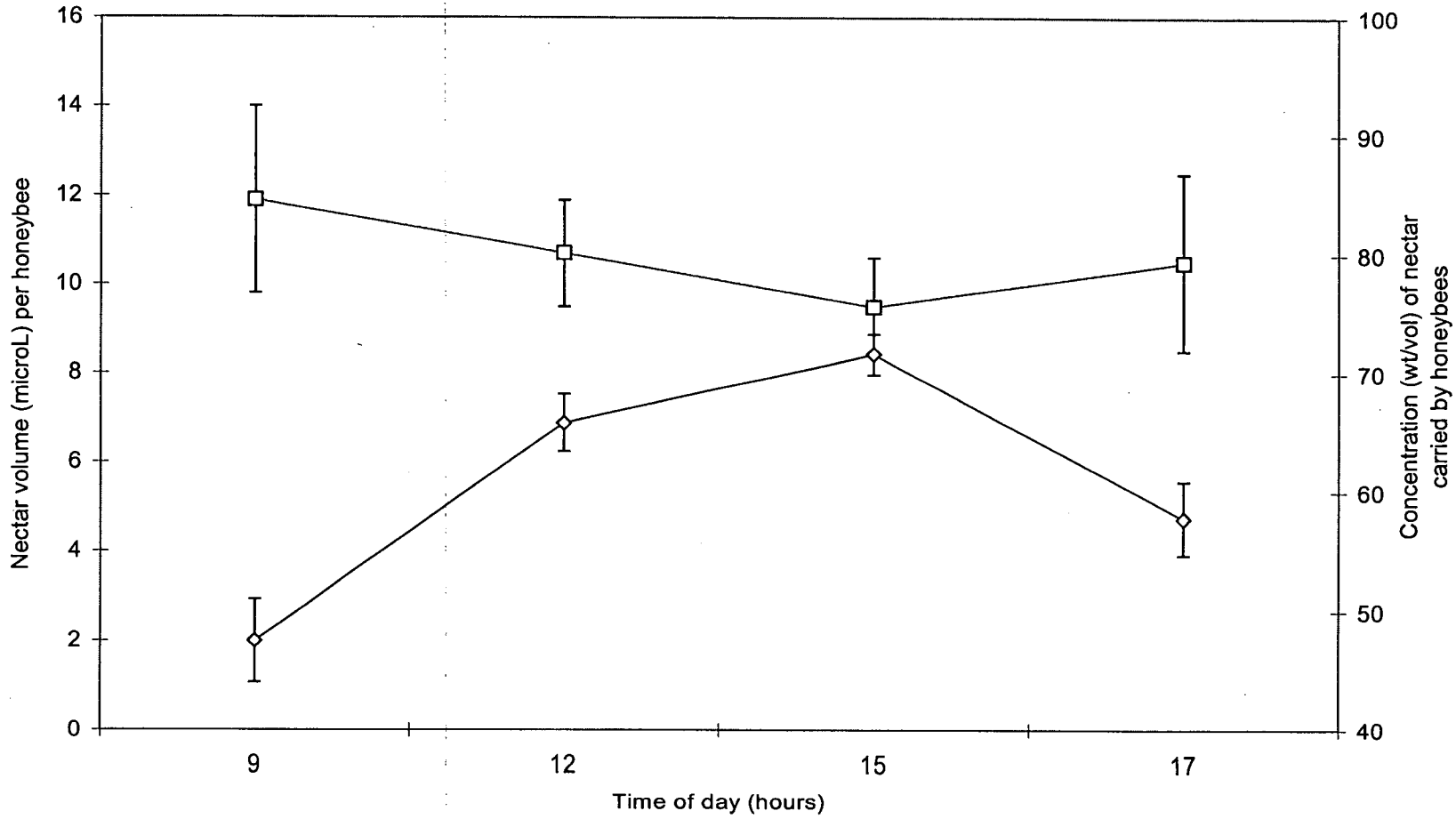

Fig. 3.3. Volume (squares) and concentration (diamonds) of nectar carried by honeybees returning to the hive over the day. Error bars are standard errors.

Table 3.5. The mean weight of nectar sugar available per flower, the amount of sugar removed per honeybee visit (estimated at 50% of available sugar), the mean±se sugar weight carried by returning bees, and the number of flowers required to make up this returning load at 0900, 1200, 1500 and 1700 hours. The number of trees this would require, assuming 9 flowers are visited per tree, is also shown.

Time of day	Sugar weight /flower (mg)	Sugar removed/visit (mg)	Sugar weight/bee (mg)	No. flowers	No. trees
0900	0.90	0.45	5.52±0.98	12.3	1.4
1200	0.80	0.40	7.07±0.82	17.7	2.0
1500	0.41	0.21	6.62±0.76	31.5	3.5
1700	0.52	0.26	6.49±1.45	25.0	2.8

familes) as well as the predominant native visitor to flowers, followed by coleopterans and native bees and wasps. *E. lucida* would therefore appear to be a broad generalist in terms of its flower visiting fauna. However, the importance of a pollinator depends on factors other than visitation rate (Schemske and Horvitz 1984; Ashman and Stanton 1991). Of the native insects visiting flowers, only the larger dipterans (principally tachinids, tabanids, calliphorids and muscids), large coleopterans (principally mordellids, cantharids and lycids), and native bees and wasps (principally sphecids, ichneumonids and colletids) appeared to be effective pollinators of *E. lucida* flowers.

Large dipterans (≥5 mm) typically foraged for nectar by perching on the massed stamens and probing downwards to the nectaries, resulting in moderate pollen loads on the insects' ventral surface and a high incidence of stigma contact. In contrast, small dipterans tended to skirt around the edge of the flower while accessing nectar from the base of the dense mass of stamens. Stigma contacts were relatively infrequent for small dipterans and these flies carried very little pollen. Beardsell *et al.* (1993) also found that the most frequent visitors to flowers of *Thryptomene calycina*, the microdiptera (flies <5 mm), carried no pollen on their bodies and were ineffectual as pollinators, while larger blowflies carried abundant pollen and were important cross-pollinators of *T. calycina.* Similarly, Williams and Adam (1998) found that larger dipterans captured on flowers of subtropical rainforest trees carried variable but often substantial pollen loads.

Large coleopterans (mainly mordellids and lycids) also tended to alight and feed on the massed stamens of *E. lucida* flowers leading to frequent stigma contact, while small coleopterans fed on nectar around the flower edge well away from both pollen and stigma. Large coleopterans carried significant amounts of *E. lucida* pollen on their ventral surface. Williams and Adam (1998) also found large (>9 mm) beetles feeding on flowers of subtropical rainforest trees carried variable but frequently substantial loads of pollen. Similarly, House (1989) found coleopterans carried the most pollen of all visitors to several species of tropical rainforest trees, and that beetles were likely to be the most important pollinator for at least one of the species.

In addition to native dipterans and coleopterans, flowers of *E. lucida* were visited by native colletid bees, as well as by native sphecid and ichneumonid wasps. However, native Hymenoptera made up a minor component of the anthophilous fauna of *E. lucida*, and appear to be secondary in importance as pollinators of *E. lucida* flowers after native flies and beetles.

E. lucida flowers supported a range of squatter insects, primarily thrips, small beetles (mainly staphylinids in the subfamily Aleocharinae) and predatory

spiders. Thrips are pollen feeders which may also consume nectar (Kevan and Baker 1983), while the majority of small beetles were observed feeding on both nectar and occasionally pollen. Squatter insects are generally considered to be too small to make regular contact with the flower stigma, and tend to remain within individual flowers or branches, preventing them from carrying out cross pollinations (Lloyd and Schoen 1992). However, evidence from bagging, emasculation and insecticide treatments (see Chapter 2) indicates that squatters may effect low levels of self pollination in *E. lucida* flowers. Baker and Cruden (1991) also found that thrips and aphids were responsible for significant amounts of self pollination in flowers of *Ranunculus scleratus* and *Potentilla rivalis* as they crawled around flowers.

Variation in pollinator assemblage and visitation rates

The relative importance of different pollinators can vary in space, between years, and over a single season (Motten *et al.* 1981; Ashman and Stanton 1991; Ollerton 1996; Fishbein and Venable 1996). I ranked the relative importance of the major pollinator groups of *E. lucida* over fourteen sites, between two different years for three of the sites, and over a season for one site. The introduced honeybee dominated at all sites (both near to and >2 km from commercial apiaries), in all years and throughout the season, and clearly forms an important and ubiquitous component of the insect fauna visiting *E. lucida* flowers. The relative importance of the native pollinators also tended to be consistent, with large dipterans the most important native pollinators in 17 out of 19 cases (Table 3.4). Similarly, the composition and relative importance of native pollinators were generally similar between years at the Link Road (Table 3.4), despite the introduction of commercial hives to two of the sites and large variation in visitation rates between years (Table 3.3). In contrast, Fishbein and Venable 1996) observed a major temporal shift in the relative 'pollinator effectiveness' of different pollinators to *Asclepias tuberosa* due to changes in visitation rates between consecutive years.

The ranking of native pollinators of *E. lucida* changed considerably over a season at MAY, with coleopterans dominating in early and mid season and large dipterans the dominant visitor in late season (Table 3.4). Ashman and Stanton (1991) also recorded a significant shift in the relative abundances of different pollinators of *Sidalcea oregana* ssp. *Spicata* over a season. Because different pollinators varied significantly in their relative efficiencies at pollinating flowers, Ashman and Stanton (1991) sugested that seasonal shifts in abundance may also have been accompanied by changes in pollen receipt by *S. oregana* flowers.

While the composition and relative importance of the native pollinators was generally consistent between sites and years, total visitation rates varied by greater than an order of magnitude. The reason for the very wide variation in visitation frequency between different sites is not known. It is unlikely to be due to insects foraging for nectar on other flowering plants as *E. lucida* is the single predominant source of nectar available in cool temperate rainforest during the summer months (see Chapter 7). The number of large flies caught on sticky traps also varied widely between sites (see Chapter 8), indicating that the variation in visitation rates between sites reflects variation in the absolute abundance of insects in the forest and not just in their behaviour at *E. lucida* flowers. This variation in insect abundance and activity occurred despite the ostensible similarity of the rainforest sites, did not appear to be related to altitude or aspect, and was evident over a relatively small scale. For example, sites WAR3 and WAR6 were in a contiguous area of rainforest separated by only 2 km, were sampled on the same days, yet had visitation rates of 7.0 and 2.7 visits per flower per day, respectively (Table 3.3).

In addition to site-to-site variation, visitation rates also varied substantially between years and over a season (Table 3). A further source of variation in pollinators of *E. lucida* also occurs as a result of the weather patterns characteristic of western and southern Tasmania. Cold fronts embedded in the 'Roaring Forties' airstream regularly cross Tasmania's west and south, bringing rapid drops in temperature and high precipitation (Jackson 1999). These spells of cold and wet weather occur intermittently even during the summer months, and can result in substantial variation in insect activity within and between days (see Chapter 1).

From the plant's perspective, such spatial and temporal variation in insect abundance and/or activity represents a significant problem in ensuring reproductive success under highly variable and unpredictable conditions of pollinator service. Much of the pollination ecology of *E. lucida* appears to reflect adaptation to cope with this variability in pollinators. Extended anthesis provides one means of maximising mating opportunities in a variable environment (Motten *et al.* 1981; Motten 1983), and *E. lucida* flowers are relatively long lived (*ca.* 12 days), with a 6 day male-phase followed by 6 days of stigma receptivity (see Chapter 2). *E. lucida* flowers are also partially self-fertile, and are capable of autogamously setting fruit in the absence of visitors. Furthermore, the unspecialised dish-bowl type blossom and concentrated nectar of *E. lucida* flowers make them highly attractive and accessible to a broad taxonomic and size range of insects. While only a portion of the total visitors to flowers are capable of efficiently pollinating flowers, *E. lucida* nevertheless has

a generalised pollination system employing several orders and at least 10 common insect families as potential pollinators. Such a generalized pollination system is likely to be advantageous in a species which experiences significant temporal and/or spatial variance in pollinator service (Waser *et al.* 1996; Johnson and Steiner 2000).

Honeybees as pollinators of E. lucida

Honeybees appeared to be efficient pollinators of *E. lucida* flowers, at least from the perspective of female function (i.e. pollen deposition). Honeybees carried substantial loads of *E. lucida* pollen on their ventral surface and foraged in flowers in such a way that the transfer of pollen onto stigmas was highly likely during any one visit. Preliminary tests on the deposition of pollen by honeybees during visits to virgin emasculated flowers indicated that honeybees deposited 5.14±.2.46 (n=9) pollen grains per visit to emasculated flowers, with 97.7% of these deposited grains belonging to *E. lucida*.

However, the efficiency of a pollinator depends not only on the frequency of visits and number of grains deposited, but also on the quality of pollen, primarily on whether it is cross or self (Motten *et al.* 1981; Price and Waser 1982; de Jong *et al.* 1993). In a mass-flowering, hermaphroditic species such as *E. lucida*, the relative proportions of cross and self pollen deposited during a honeybee visit will be a function of the number of flowers visited on a plant during a visitation sequence (Klinkhamer and de Jong 1993), as well as the extent of pollen carryover between flowers (Thomson 1986; Robertson 1992) and the position of the flower in the visitation sequence (Thomson and Plowright 1980; Price and Waser 1982). As an extreme example, Paton (1997) followed individual honeybees foraging on *C. rugulosus* plants and recorded over 4600 visits over 9.9 hours without observing a single inter-plant movement by a honeybee.

I found that honeybees foraged on a limited number of *E. lucida* flowers per tree during a visitation sequence (on average *ca.* 9 flowers per tree). A number of other studies have also found that insects visit only a small percentage of flowers on a plant during a visitation sequence (e.g. Frankie *et al.* 1976; Haynes and Mesler 1984; Robertson 1992; Klinkhamer and de Jong 1990; Klinkhamer *et al.* 1994; also Lloyd and Schoen 1992). The reasons why honeybees vacated trees after visiting <10 flowers are unknown, but did not appear to be related to nectar levels or total blossoms available, as honeybees visited a similarly small number of flowers per tree regardless of mean nectar levels or the total number of flowers on a tree. Similarly, tree departure did not appear to be due to satiation, as full nectar loads required a minimum of two

trees to be visited in the morning when floral nectar was highest, and as many as four trees when nectar was most scarce during the middle of the day (Table 3.5). Indeed, honeybees were frequently observed leaving one tree to forage on another tree some distance away. It is possible that visiting only a limited number of flowers per tree independent of nectar levels and flower number reflects an avoidance strategy, where remaining too long on a tree would make a foraging honeybee vulnerable to being predated. If so, the behaviour must reflect predation pressures in the honeybees' native range, as there are no significant predators of honeybees in Tasmania.

Regardless of the reasons for limiting the number of flowers visited per tree, the fact that honeybees frequently change trees presumably increases their potential for cross pollination of *E. lucida* flowers. The principal native pollinators (large flies) also appeared to visit only a limited set of flowers on a tree before leaving, and honeybees may resemble large native flies in their pollination capabilities. Data on fruit and seed set at apiary and non-apiary sites indicate that the presence of increased numbers of honeybees has little net effect on female reproductive success of *E. lucida* trees (see Chapter 9), indicating that hive honeybees may in fact be providing a comparable pollinator service to that afforded by the native insect and feral honeybee fauna.

Chapter 4. Facultative dichogamy and reproductive assurance in partially protandrous plants

Following the pioneering work on pollination biology of the late eighteenth and nineteenth centuries, the phenomenon of dichogamy (the separation of presentation of pollen and stigmas in time) was, together with a plethora of other floral features, interpreted exclusively as an adaptive mechanism enhancing outcrossing. More recently however, David Lloyd and co-authors (Lloyd and Yates 1982; Lloyd and Webb 1986) have highlighted the inconsistency of the outcrossing argument in species which possess both dichogamy and one or more additional mechanisms (strong self-incompatibility, unisexuality, herkogamy) which preclude or limit opportunities for self-fertilization. Lloyd *et al.* elegantly resolved this dilemma by viewing dichogamy primarily as a mechanism that reduces interference between the sexual functions of pollen export and receipt, while accepting that dichogamy will also often reduce self-fertilization.

Dichogamy may take two forms, with stigma presentation preceding (protogyny) or following (protandry) the presentation of pollen (Lloyd and Webb 1986). Which form of dichogamy a plant practices has important implications for the plant's effectiveness in avoiding self-interference and self-fertilization. The efficiency with which the respective modes of dichogamy avoid self-interference depends primarily on the relative ease of moving androecia and gynoecia (Lloyd and Yates 1982; Lloyd and Webb 1986; Webb and Lloyd 1986; see below). In contrast, the effectiveness of the two modes of dichogamy in preventing self-fertilization depends on the degree of dichogamy, and on which 'mode' of selfing is encouraged (Lloyd and Schoen 1992). Where dichogamy is complete, self-fertilization is totally precluded irrespective of the order of stigma/anther presentation (Lloyd and Webb 1986). However, many species exhibit some overlap in presentation (partial dichogamy), and it is here that differences in the efficiency of preventing selfing and the selfing mode become apparent.

In incompletely protogynous flowers, there is a period of stigma receptivity (and opportunities for outcrossing) before selfing is possible, with delayed selfing the result (Lloyd and Webb 1986; Lloyd and Schoen 1992; Lloyd 1992). In contrast, in flowers which are incompletely protandrous, selfing can occur as soon as cross-fertilization is possible, unless all pollen has been previously removed (Lloyd and Webb 1986). The result is competing selfing (simultaneous opportunities for cross- and self-fertilization; Lloyd and Schoen 1992), although strictly speaking selfing may be prior (where opportunities for selfing precede

opportunities for outcrossing) if visitation rates are very low. Because delayed selfing allows cross-fertilization to occur before selfing, entails no pollen or seed discounting, and ensures all un-crossed ovules are selfed late in the flower's life, this mode of selfing is always selectively advantageous (Lloyd 1979, 1992). Competing and prior selfing can provide reproductive assurance; however, both selfing modes are subject to pollen and seed discounting (Lloyd and Schoen 1992), and conditions favoring competing and prior modes are more stringent (Lloyd 1979). Therefore, where the aim is total avoidance of self-fertilization, complete dichogamy is preferred, although this precludes the possibility of reproductive assurance through selfing. Where plants are partially dichogamous, self-fertilization is effectively reduced while still permitting some selfing. However, of the two possible modes of dichogamy, partial protogyny should be more advantageous than partial protandry in both avoiding un-wanted self-fertilization, and in the provision of reproductive assurance through delayed selfing without the downside of pollen and seed discounting.

Why then is protandry so common in insect-pollinated plants? One reason, touched on above, relates to the relative ease with which the male and female organs can be removed after functioning. As noted by Lloyd and Webb (1986), separation of the male and female functions in time requires both that the second-functioning organ be kept out of the way while the first is being presented, and that the first be kept out of the way during subsequent presentation of the second. The former objective is typically achieved by delaying the growth of the second-functioning organ. For the second objective, however, it may be easier to remove the relatively flimsy stamens compared to the carpels (for example, via abscission or curvature of filaments), which would favor prior presentation of pollen (i.e. protandry). Indeed, Lloyd and Webb (1986) propose the easier removal of stamens after functioning as the principal reason behind the predominance of protandry in insect-pollinated plants.

However, the suggestion that it will be easier to remove the androecia after it has enlarged and functioned may not always be relevant. Presumably, separation can be achieved in one of two ways: by removing the first-maturing organ (in protandrous species, the relatively flimsy androecia, as above), or by retaining that organ in place and extending the subsequent organ beyond the reach of the first. Here, the relative bulk and rigidity of the gynoecium and the difficulty of its removal would not be an issue. For example, in protandrous species, extending the style and/or stigmas beyond the previously-opened anthers would achieve separation without the first-opening organ having to be moved. The same process could occur equally well in protogynous species in

which the filaments extend beyond the reach of stigmas after an initial female phase.

If, as suggested above, it is as easy to separate the sexual functions in either mode of dichogamy by extending the second-functioning organ, and partial protogyny (in contrast to partial protandry) provides both a more effective reduction in self-fertilization and reproductive assurance without discounting, then the phenomenon of protandry remains problematic. Indeed, the prevalence of this mode of dichogamy in insect-pollinated plants suggests some further selective force must be operating in favor of prior presentation of pollen.

The distribution of the 'sexes' in a hermaphrodite blossom refers purely to the two functions of export and reception of pollen (Faegri and van de Pijl 1971). Furthermore, the two sexual phases are not fixed, but depend on a variety of internal and external factors. The dependence of the pistillate phase on rates of pollen receipt and fertilization of ovules has been well documented in a range of species (Devlin and Stephenson 1984; Richardson and Stephenson 1989; Ishii and Sakai 2000). Similarly for the male function, although the initiation and rate of pollen release (ie. anther dehiscence) may be internally regulated, the period over which pollen is *actually available* in the flower is in many species an external function of visitation frequency. This stems from the fact that, in insect-pollinated plants, pollen tends to adhere to dehisced anthers until physically removed by a visitor. In protandrous species in which pollen removal is visitor-mediated, the length of time pollen remains in a flower - and therefore the extent of separation (or overlap) of the 'sexes' - will be a function of visitation frequency. That is, protandry is facultative. When insect visitors are abundant and pollen is rapidly removed, overlap in the sexual functions will be minimal or absent. When insect visitors are rare, however, pollen is retained in the flower for longer periods, leading to increased overlap in pollen and stigma receptivity, with the degree of overlap increasing with a decline in visitation rate.

It follows that in insect-pollinated, self-fertile species in which anthers and stigmas are in close proximity (ie. non- or weakly-herkogamous), facultative protandry should lead to an increased rate of autogamous deposition of self pollen and selfing of ovules when insect visitors are rare. Although such selfing is 'competing' *sensu stricto* (Lloyd and Schoen 1992), it would appear to come under the heading of 'environmentally induced selfing'– an increase in the selfing rate under poor environmental conditions that limit opportunities for outcrossing (Lloyd and Schoen 1992; Lloyd 1992). Environmentally induced selfing has been described twice in the literature. Schoen and Lloyd (1984) first reported the phenomenon in flowers (known as 'pseudocleistogamous; Lord

1981) which self-fertilise in bud under unfavorable conditions for anthesis, while Schoen and Brown (1991) described another instance in the (chasmogamous) flowers of the legume *Glycine* in a subalpine population where insect visits were rare. Both examples refer to a process of 'whole flower' selfing in which all ovules of a flower are selfed under poor conditions (Schoen and Brown 1991). In contrast, environmentally induced selfing through facultative protandry should lead to varying proportions of selfed and crossed ovules within a flower (ie. 'part-flower' selfing) depending on the frequency of insect visits, although where visits are extremely rare or absent, the result could potentially be all ovules selfed within individual flowers.

Facultative protandry represents a simple, potentially widely applicable mechanism for ensuring environmentally induced selfing in insect-pollinated plants. Nevertheless, general references to the process underlying facultative protandry (i.e. the functional link between the period of pollen presentation, overlap in the sexual functions, and visitor frequency) occur only occasionally in the literature, and detailed descriptions are rare (but see Vaughton and Ramsey 1991).

Empirical tests for the occurrence of environmentally induced selfing via facultative protandry should be relatively straightforward. In its most extreme form, flowers protected from visitors (e.g. by bagging or greenhouse-cultivation) should retain pollen well into the pistillate phase, show substantial autogamous deposition of pollen onto stigmas, and set some fruit and seed. In open flowers under field conditions, one would expect an inverse relationship between the frequency of insect visits and pollen retention. Where visits are common, all pollen should be removed before the onset of stigma receptivity (i.e. dichogamy is complete), while as visits decline, overlap in pollen presentation and stigma receptivity should increase. To test whether facultative protandry provides reproductive assurance, fruit and seed set in intact and emasculated flowers should be examined over a gradient of visitation frequency (Eckert and Schaefer 1998). Where insect visits are common and there is no overlap in pollen presentation and stigma receptivity, emasculation should have no effect on fruit/seed set. However, where visitors are infrequent and pollen is retained into the pistillate phase, emasculation should reduce fruit/seed set if environmentally induced selfing is indeed contributing to maternal reproductive success.

Although empirical evidence is so far lacking, I suggest that facultative protandry may in fact be very widespread in insect-pollinated plants and play an important, hitherto unrecognised role in floral evolution. The phenomenon can be seen to provide an alternative 'best of both worlds' strategy (Becerra and

Lloyd 1992) to that afforded by delayed selfing in partially protogynous plants. It allows maximal outcrossing (all pollen removed, no overlap in the sexual functions when visitors are common) with backup selfing of unfertilized ovules when visitors are uncommon, and incurs minimal seed and pollen discounting when crossing is least likely.

Furthermore, if environmentally induced selfing via facultative protandry provides comparable advantages to that afforded by delayed selfing in protogynous plants, the phenomenon may help to explain the predominance of protandry among insect pollinated plants. Because the 'natural' developmental progression in plant appendages (including flowers) is centripetal (ie. from the outer to the inner whorls), protandry represents the original or ancestral condition and protogyny a derived state (Faegri and van de Pijl 1971). If the two possible modes of dichogamy provide comparable selective advantages, as suggested above, then one might expect the more primitive condition, protandry, to predominate. In this context, protogyny would represent a specific, derived condition in response to particular conditions in which protandry is unable to provide a comparable selective advantage.

One condition in which protandry is likely to be inferior to protogyny may occur where the pollinating agent is abiotic, which in turn may explain the overwhelming predominance of the protogynous condition in wind-pollinated species (Lloyd and Webb 1986). In a protandrous, wind pollinated species, the positive functional relationship described above between the rate of pollen removal and the likelihood of outcrossing is likely to be much weaker than in insect pollinated plants. In the absence of a strong facultative link between pollen/stigma separation and the likelihood of outcrossing, the protandrous condition should therefore suffer from its inability to provide reproductive assurance without seed and pollen discounting (*cf.* Faegri and van de Pijl 1971; Lloyd and Webb 1986). Under such conditions, the derived condition of protogyny may represent a more advantageous option in the avoidance of unwanted selfing and the provision of reproductive assurance without discounting.

Chapter 5. Patterns of nectar and pollen release in flowers of *E. lucida*: effects on male and female fitness

Abstract

Flowers of *E. lucida*, are hermaphroditic and protandrous, with a 6-7 day male phase followed by 6 days of stigma receptivity. Nectar is produced throughout anthesis. The rate of nectar production is independent of temperature, and flowers do not reabsorb accumulated nectar sugar. Dehiscence of the *ca.* 80-120 anthers is staggered over the first few days of anthesis and most pollen is removed before stigma receptivity begins. However, results of field tests on anther dehiscence over a range of ambient conditions indicate that the rate of dehiscence is strongly dependent on temperature. Release of pollen was minimal below around 10°C, while above 10°C dehiscence increased linearly with increasing ambient temperature. On warm days when insects are active, flowers of *E. lucida* steadily release nectar and pollen, while on cold days when insects are inactive, nectar accumulates rapidly while the release of pollen is retarded. The result is a highly responsive array of nectar-presentation patterns and pollen-release schedules which maximise both male and female function under a wide range of pollinator-abundance and weather conditions.

Introduction

Bateman (1948) first pointed out that in oogamous species in which the mother's investment is much greater than the father's, maternal fitness should be limited by a mother's ability to accrue resources. Paternal fitness in contrast will be limited by a male's ability to achieve fertilisations. Bateman (1948) envisioned sexual selection primarily in terms of animals with separate sexes. Extension of the concept to plants dealt initially with the evolution of separate male and female flowers on the same (monecy) or different plants (dioecy) (Wilson 1979; Bawa 1980), and to secondary sex differences between male and female flowers and plants (Lloyd and Webb 1977).

More recently, sexual selection theory has been applied to the concept that sex-differential forces may act within a single hermaphroditic flower (Lloyd and Yates 1982; Lloyd 1984; Harder and Thomson 1989). Because pollen dispersal and receipt are distinct processes, structures and behaviours that are beneficial for one sex may not coincide with what is optimal for the other sex (Lloyd and Yates 1982; Harder and Thomson 1989; Klinkhamer and de Jong 1990, 1993; Klinkhamer *et al.* 1994). Furthermore, because sexual selection operates through the agency of pollinators in insect pollinated plants (Bawa 1980), both sexual functions in hermaphroditic flowers may be affected by natural variation

in the abundance and activity of pollinators. Because such effects often differ in extent and even direction for the two sexual functions, the maximising of net fitness by a plant requires a balancing of male and female fitness under varying (and often unpredictable) pollinator conditions (Sutherland and Delph 1984).

E. lucida occurs as a canopy co-dominant in cool temperate rainforest in the wetter western and southern regions of Tasmania. Flowering commences in December and lasts for 4-6 weeks, with individual trees bearing thousands of flowers. The four-petalled flowers are white, relatively large (*ca.* 40 mm diameter), actinomorphic and hermaphrodite, with a central style and 5-7 lobed stigma surrounded by a dense whorl of approximately 80-120 stamens. *E. lucida* flowers are long lived (12 days) and protandrous, with a 6 -day male-phase during which the anthers dehisce in a staggered pattern followed by 6 days of stigma receptivity (Chapter 2). The distinctly-flavoured nectar secreted at the base of the stamens is produced throughout anthesis, with similar quantities of nectar produced by male- and female-stage flowers (Ettershank and Ettershank 1992; Chapter 1). Rates of nectar production are independent of temperature and visitation rate (Chapter 1), and nectar rapidly accumulates in flowers at sites where insects are scarce and visitation rates restricted (Chapter 8) and on cold days when insects are inactive (Chapter 1). Nectar is the sole attractant for the dominant pollinators, large (≥5 mm) native flies in the families Muscidae, Calliphoridae, Tabanidae and Tachinidae, although other potential pollinators (syrphid flies, larger beetles and native bees) also take pollen during visits (see Chapter 3).

E. lucida is partially self-compatible. Flowers exposed to pollinators set high levels of fruit with relatively low percent seed set, while fruit and seed set in bagged flowers (autogamously self-pollinated) is substantially reduced (Ettershank and Ettershank 1992; Chapter 2). *E. lucida* flowers do not appear to be pollen limited, at least from the perspective of number of grains deposited on stigmas. At sites where insect visitors are frequent, individual stigmas receive a super-abundance of pollen grains (estimated at >1700 grains/stigma) relative to the number of ovules per ovary (n=40; Chapter 2). Even at sites where visitors are extremely infrequent (as low as 2 visits/flower/day), flowers receive comparable quantities of pollen on stigmas (Chapter 9), apparently as a result of autogamous deposition in flowers in which pollen has been allowed to accumulate into the female phase (Chapter 2).

The cool temperate rainforest environment in which *E. lucida* grows is characterised by both temporal variability in pollinator activity due to the intermittent cold and wet weather in summer, and by substantial spatial heterogeneity in pollinator abundance (Chapter 3). As part of a 3-year study into

the pollination ecology and breeding system of this cool temperate rainforest tree, I investigated aspects of pollen and nectar production in flowers of *E. lucida*. This chapter reports on rates of anther dehiscence in the field over a range of ambient temperatures, and interprets patterns of nectar and pollen release in *E. lucida* flowers in terms of optimising male and female fitness under varying pollinator conditions.

Methods

I examined the role of temperature in regulating pollen release in January 1999 at MAY (see Study Sites section).

In examined the effect of temperature on rates of anther dehicence over 12 days of varying ambient conditions in January 1999. On each day I tagged a total of 10-15 flowers spread over 1-3 branches of a tree between 0900 and 1000 hours by tying a small loop of colored wire to the flower pedicel. Different trees were used on different days. All tagged flowers were in early-mid male phase (5-30% of anthers dehisced). I then removed all pollen from the tagged flowers by snipping off any white anthers (i.e. those with pollen adhering) with fine scissors. At this 'zeroed' stage, all anthers were either pinky-red (un-dehisced) or brown (dehisced and pollen dislodged). The branches with tagged flowers were than bagged with netting and left for 6 hours, after which the bags were removed and the number of white anthers on the tagged flowers (i.e. those that had dehisced since bagging) were counted. At three times during the bagging period (at bagging, after 3 hours and on removal of bags) I recorded the temperature and humidity inside the bags using an electronic hygrometer and used the mean value of the three readings as an index of the temperature/humidity over the 6-hour bagging period. The ranges in mean temperature and mean humidity for the twelve 6-hour bagging periods were 9.5 to 24.7°C and 33.0 to 83.3%., respectively.

Results

There was a highly significant relationship between mean temperature during the 6-hour bagging period and the rate of anther dehiscence (Fig. 5.1). Anther dehiscence was minimal below a mean temperature of around 10°C. Above this cut-off point, rates of dehiscence increased linearly with mean temperature (Fig. 5.1). The line was described by the equation: Number of anthers dehisced/6-hours=1.29(mean temperature)-12.92 ($F_{1,10}$=24.41, r^2=0.71, P<0.001). There was no indication that the line had begun to level off by the highest mean temperature recorded (24.7°C). At this mean temperature, anthers dehisced at a rate of 25.4 anthers/6-hours or an average of 4.2 anthers/hour (Fig. 5.1). There

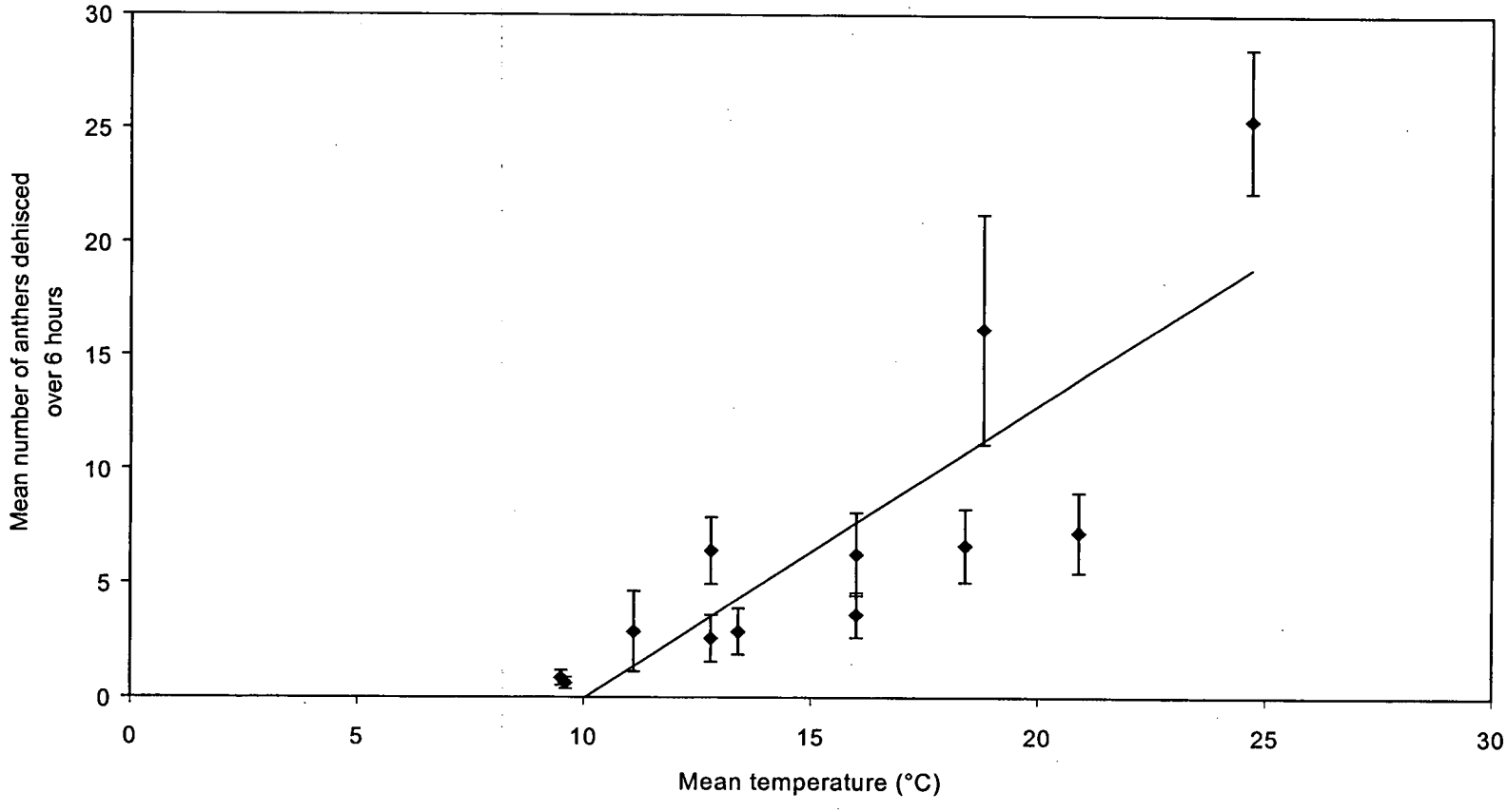

Fig. 5.1. Changes in the rate of anther dehiscence with changes in ambient temperature. Linear regression line is shown. Error bars are standard errors. n=10-15 for all points.

was also a weak negative relationship between rate of anther dehiscence and mean humidity ($F_{1,10}=3.79$, $r^2=0.28$, $0.05<P<0.1$). Because ambient temperature and humidity negatively covary (the regression of humidity on temperature was significant, $F_{1,10}=5.76$, $r^2=0.37$, $P<0.05$), it is difficult to partition the effects of the two variables. However, the relationship between dehiscence and temperature was much stronger than between dehiscence and humidity. Furthermore, because temperature tends to drive changes in humidity, rather than vise versa, temperature is probably the principal determiner of rates of dehiscence in *E. lucida* flowers. Humidity however may have a secondary effect on rates of dehiscence, further reducing the rate at which pollen is released when conditions are cold and wet (i.e. close to saturation), and dampening rates of dehiscence when conditions are warm and humid.

Discussion

Sexual selection and maximising flower fitness

Where a perfect-flowered plant is not pollen limited, the optimal design and functioning of flowers in attracting pollinators should rest primarily with male function (Lloyd 1984; Pleasants and Chaplin 1983; Harder and Thomson 1989). Gains in paternal fitness could be achieved through a variety of mechanisms effecting pollen removal, transport and deposition on the stigmas of recipient flowers (Harder and Thomson 1989; Harder and Wilson 1994). In particular, the methods by which flowers deploy pollen can effect the efficiency of pollen export (Harder and Thomson 1989). For example, the release of pollen in small packages and its dispersal via multiple visitors effectively maximises the proportion of pollen reaching stigmas, given that successful pollen export is typically a decelerating function of the amount presented (i.e. diminishing returns; Harder and Thomson 1989; Thomson *et al.* 1989; Harder 1990; Harder and Wilson 1994). Distributing pollen over many different visitors should also act to spread the risk of pollen loss between multiple vectors (Lloyd and Yates 1982; Harder and Thompson 1989).

Structural mechanisms for limiting the amount of pollen exposed to a pollinator include packaging mechanisms, most commonly the staggering of anther dehiscence within flowers or inflorescences, or dispensing mechanisms which restrict the amount of pollen removed on a single visit (Harder and Thomson 1989). Of the two, packaging mechanisms may be more advantageous as they allow facultative responses to variation in pollinator abundance. This occurs through the simple process of pollen accumulating on anthers in the absence of visits, leading to an increase in the amount of pollen presented (and removed) with increasing time since the last visit (Harder and Thomson 1989).

A further, non-structural, mechanism allowing facultative responses to pollinator abundance involves nectar production (Harder and Thomson 1989; Harder and Wilson 1994). In the first instance, the continuous presentation of small quantities of attractive (i.e. concentrated) nectar should maximise the number of short-duration visits to flowers (Harder and Thomson 1989; Thomson *et al.* 1989; see Chapter 1). Furthermore, if this nectar is allowed to accumulate in the absence of visitors (e.g. Kadmon 1992; Thomson *et al.* 1989), the duration of pollinator visits (e.g. Thomson and Plowright 1980; Thomson 1986) and the amount of pollen removed (e.g. Harder 1990; Young and Stanton 1990) should increase with the interval since the flower was last visited. Such a mechanism effectively increases removal per visit when visits are infrequent and allows for a facultative modification of removal with changes in visitation rate (Harder and Wilson 1994).

Deposition of pollen on stigmas also depends on the length of flower visits, so the patterns of pollen and nectar release may also have an impact on female fitness of hermaphrodite flowers (Harder and Thomson 1989). However, the influence of female fitness on selection for optimal schedules for nectar and pollen release will depend on the degree to which fruit and seed set are pollen limited (Lloyd 1984; Sutherland and Delph 1984; Harder and Thomson 1989), either through the quantity or quality of deposited pollen (Ramsey and Vaugton 2000).

Optimal dispensing schedules for pollen and nectar in E. lucida *flowers*

E. lucida flowers are not pollen limited, at least from the perspective of number of grains deposited on stigmas, and maximising male fitness may be the predominant consideration determining optimal patterns of nectar and pollen release (Lloyd 1984). However, although stigmas receive a superabundance of pollen, flowers may be pollen limited from the perspective of pollen quality (Ramsey and Vaughton 2000) as much of the pollen deposited in flowers is likely to be self pollen (see Chapters 2 and 9). Patterns of nectar and pollen release may therefore also have an influence on female fitness, particularly with respect to the proportion of self pollen (both autogamous and geitonogamous) deposited on stigmas.

Details of the pollination ecology of *E. lucida* fit reasonable well with Harder and Thomson's (1989) model of a facultative dispensing schedule for nectar and pollen release which maximises male fitness. Flowers have numerous anthers which sequentially dehisce over a number of days (i.e. pollen packaging), and pollen builds up on anthers in the absence of visits (see Chapter 2). Flowers secrete a relatively dilute nectar (*ca.* 20% sugar wt/wt) which on

warm dry days is rapidly concentrated to >60% sugar wt/wt (Chapter 3). This concentrated nectar is highly attractive to insects and flowers can receive >20 visits per day from potential pollinators (Chapter 3). At sites where insects are scarce and visits to flowers are rare, nectar rapidly accumulates in flowers (Chapter 8). Similarly, because nectar production is independent of temperature, production rates are undiminished on days when insect activity is depressed due to cold weather. For example, nectar accumulated in *E. lucida* flowers on a single cold day (temperature maximum <15°C) at a similar rate to that in bagged flowers on warm days when insects were active (see Chapter 1). *E. lucida* flowers with abundant nectar are highly attractive to both native insects and introduced honeybees, with the latter spending longer in flowers with greater accumulations of nectar sugar (see Chapter 3).

A dependence of the rate of anther dehiscence on temperature introduces an additional variable into the above model of optimal schedules for nectar and pollen release. I suggest that this linking of rates of anther dehiscence to temperature, coupled with temperature-independence of nectar production, represents a response to substantial temporal (weather) and spatial variation in pollinator conditions.

E. lucida experiences stochastic variation in pollinators on two quite different scales. Cold fronts embedded in a powerful westerly airstream (the 'Roaring Forties') cross Tasmania from the west at intermittent intervals year-round, and cold spells lasting 1-3 days can be common events even in mid-summer (Jackson 1999). Insect activity is minimal during these cold snaps, and they represent significant and unpredictable interruptions in pollinator service to *E. lucida* during anthesis of flowers. Added to this temporal variability is a pronounced and apparently stochastic spatial heterogeneity in the absolute abundance of pollinating insects in cool temperate rainforest, with as much as an order of magnitude difference in visitation rate to flowers between ostensibly similar sites (Chapter 3).

These two types of unpredictability in pollinators involve quite different risks to *E. lucida* flowers. For example, an absence of visitors during intermittent cold spells represents only a temporary deficiency at sites with abundant insects, a deficiency which is removed as soon as the weather clears. In contrast, an absence of visitors at sites with few insects is likely to represent a long-term deficiency, regardless of weather conditions. Given that both forms of variation are stochastic and presumably cannot be predicted by individual flowers/trees, patterns of nectar and pollen release should therefore maximise flower fitness under as many weather/insect-abundance scenarios as possible.

The interaction of the two pollinator variables (weather warm/cold and insects abundant/scarce), the responses of flowers in patterns of pollen and nectar presentation, and the likely result of these patterns for pollen deposition (female fitness) and removal (male fitness) are illustrated schematically in Table 5.1.

Insects abundant-weather warm. Flowers appear well suited to maximising visits under optimal conditions, i.e. at sites with abundant insects when weather conditions are warm (Table 5.1, top-left box). On sequential warm days, anther dehiscence is staggered over the first 4-5 days of anthesis; where visitors are common, this gradually released pollen is continually removed by multiple visitors with small quantities of pollen presumably removed per visit. Such a visitation pattern closely follows the model of Harder and Thomson (1989; also Harder and Wilson 1994) and should minimise the effects of diminishing returns on male fitness. Furthermore, because insects typically visit multiple flowers on a tree (see Chapter 3), the presentation and removal of small amounts of pollen per visit should also reduce carryover of this (self) pollen to other flowers on the same tree (i.e. geitonogamy; Robertson 1992). Where selfing leads to increased abortion of seeds (seed discounting), as occurs in *E. lucida* (Chapter 2), reductions in geitonogamy should confer a significant advantage to female fitness (Hessing 1988; Carpenter 1976; Augspurger 1980; de Jong *et al.* 1993). Because high levels of geitonogamous selfing may also reduce male fitness in self-fertile plants with inbreeding depression via discounting of pollen (Harder and Barrett 1995), reducing geitonogamy may also confer a significant advantage to male function as well (Klinkhamer and de Jong 1993).

Insects abundant-cold spell. During one or more cold days (Table 5.1, bottom-left box), nectar sugar rapidly builds up in flowers, making them highly attractive to insects when the weather clears. Subsequent visits are likely to be longer and to deposit more pollen (Thomson and Plowright 1980; Thomson 1986; Klinkhamer and de Jong 1990) which may be advantageous for female fitness, particularly after several cold days in succession during which female-stage flowers will have received little pollen (Motten 1983; see also Chapter 1). In contrast, because anther dehiscence is retarded during colder weather (Fig. 1), flowers will contain relatively limited amounts of pollen. At first sight, such a result seems to contradict the facultative model of Harder and Thomson (1989) in which pollen build-up and subsequent removal increases with time since the last visit. However, because *E. lucida* flowers are long lived and high

Table 5.1. Interaction of the two variables (weather and insect abundance) affecting pollinator abundance/activity, showing the responses of flowers in nectar and pollen release, insect visitation patterns, and their effects on the deposition and removal of pollen.

Weather/Insects	LOTS	FEW
WARM	Small amounts of concentrated nectar	Rapid build-up of nectar sugar
	Small amounts of pollen	Rapid build-up of pollen
	Many short visits	**Few long visits**
	Small amounts of pollen deposited	Large amounts of pollen deposited
	Small amounts of pollen removed	Large amounts of pollen removed
COLD	Rapid build-up of nectar sugar	Rapid build-up of nectar sugar
	Limited build-up of pollen	Limited build-up of pollen
	Subsequent long visits	**Subsequent long visits**
	Large amounts of pollen deposited	Large amounts of pollen deposited
	Small amounts of pollen removed	Small amounts of pollen removed

rates of visitation immediately resume when the weather clears, it may be more advantageous for flowers to delay dehiscence and pollen removal until after a cold spell. This is because, at sites with abundant visitors, the risk of a flower being left with un-removed pollen is likely to be very low despite occasional periods of cold weather when visitation rates decline to zero. The presentation and pickup of small quantities of pollen after a cold spell (despite long visits) should also act to reduce geitonogamous transfer of self pollen between flowers visited on the same tree, further advantaging both female and male function (as above).

Insects scarce-weather warm. E. lucida trees growing at sites where the absolute abundance of insects is very low face a more intractable problem in both obtaining pollinators, and in dispensing nectar and pollen in such a way as to maximise male and female fitness when visits do occur. During fine weather (Table 5.1, top-right box), there is a rapid build-up of both nectar and pollen. Visits, when they do occur, are likely to be extended in length while the insect consumes the abundant nectar (see Chapter 3), leading to increased deposition in female-stage flowers and the removal of large quantities of accumulated pollen in male-stage flowers. This increase in the amount of pollen presented and removed with time since last visit should advantage male fitness at sites with low numbers of pollinators, given the low probability that flowers will receive another visit even where weather conditions remain favourable. Such a dispensing schedule closely follows the facultative model of Harder and Thomson (1989; also Harder and Wilson 1994) in which pollen accumulates on anthers in the absence of visits. Similarly, substantial pollen deposition in female-stage flowers may make-up for 'down-time' during cold weather, and be advantageous given the low probability that flowers will receive visits even where the weather is favourable.

Acting against the above benefits is a potential increase in geitonogamy as more pollen is picked up per visit and carried over to flowers subsequently visited on the same tree. Presumably, the potential negative effects of an increase in pollen and/or seed discounting as a result of such geitonogamous selfing are outweighed by the net benefits to male function (through facultative adjustment of pollen removal to visitation rate), and female function (through an increase in the amount of pollen deposited) when visits to flowers do occur.

Insects scarce-cold spell. Intermittent cold spells at sites with low levels of insects (Table 5.1, bottom-right) are likely to further exacerbate the difficulty of flowers obtaining sufficient pollinators. Furthermore, because cold temperatures

retard the dehiscence of anthers, flowers will contain relatively small quantities of pollen following cold weather. If a flower receives a visit following cold conditions, the insect will spend longer at flowers (due to abundant nectar) and deposit larger amounts of pollen, advantaging female function in an environment where the probability of subsequent visitation is very low. The removal of small amounts of pollen per visit should also reduce self-pollen carryover to other flowers on the same tree, minimising geitonogamous costs to female function through seed discounting. However, low absolute rates of visitation and limited pollen removal when visits do occur after cold weather both act to increase the retention of pollen in flowers. Where visitation rates are very low, large amounts of pollen may in fact be retained in *E. lucida* flowers throughout anthesis (see Chapters 2 and 9). Such retained pollen is 'wasted' in the sense of being unavailable for export to other flowers (Inouye *et al.* 1994), potentially entailing a significant cost to male fitness (Harder and Thompson 1989).

However, balancing this potential cost is the likelihood that retained pollen is available for autogamous selfing. *E. lucida* flowers are weakly herkogamous and the potential for autonomous selfing (i.e. passive within-flower transfer of self pollen from anthers to stigma) and facilitated selfing (i.e. within-flower transfer of self pollen from anthers to stigma via the action of a visitor; Lloyd and Schoen 1992) is very high (Chapter 2). Where flower visits are infrequent, there is a build-up of pollen on anthers, with an increasing overlap between pollen presentation (i.e. male phase) with stigma receptivity (female phase) with a decline in visitation rate. This breakdown in dichogamy has been termed 'facultative protandry', and the self-pollination which occurs as a result is an example of 'environmentally induced' selfing (see Chapter 4). Environmentally induced selfing occurs where there is an increase in the selfing rate under poor environmental conditions that limit opportunities for outcrossing (Lloyd 1992; also Schoen and Lloyd 1984). Like 'delayed' selfing in protogynous plants (where selfing occurs only after an opportunity for cross-pollination), facultative protandry and environmentally induced selfing should be selectively advantageous wherever pollinator numbers are inadequate for full cross-fertilisation of all ovules in a flower (Chapter 4). In this context, the retarding of dehiscence during cold weather at sites with low abundance of pollinators (Table 5.1, bottom-right), although potentially removing pollen from the export-pool, may counteract such a cost through increased rates of autogamous selfing.

Part 2. Impacts of hive honeybees

Chapter 6. Interference between native insects and honeybees at flowers of *E. lucida*

Abstract

The introduced honeybee may impact on native pollinators through either exploitation competition, or through direct interference. I used video cameras to observe direct interactions between insect visitors to flowers of *E. lucida* at sites in the vicinity of commercial honeybee apiaries and at sites >2 km from apiary sites. From 199 hours of video footage I observed a total of 1402 insect visits, 552 (39.4%) by honeybees and 850 (60.6%) by native insects (primarily native flies). I recorded a total of only 35 interactions between insects at flowers, 15 (42.9%) of which were between a honeybee and native insect. All honeybee-native interactions resulted in the displacement of the native insect visitor. There tended to be more honeybee-native interactions near commercial apiaries compared to sites >2 km from an apiary, although the difference was not significant. Given the low rate of interactions between honeybees and native insects and the super-abundance of unoccupied flowers on *E. lucida* trees, interference competition between feral and hive honeybees and native insects is likely to be minimal.

Introduction

One species may compete with another species indirectly by reducing the abundance of a common resource that is in short supply (exploitation competition), or by directly interfering in some way with the species' ability to obtain such resources (interference competition) (Schoener 1974). The former has received substantial theoretical attention over the last 50 years and has been empirically demonstrated in a wide range of plant and animal groups (Schoener 1983). However, direct interference between animals is also probably a common phenomenon in nature (Morse 1977). Interference competition has been described in a range of invertebrate and vertebrate species (e.g. Johnson and Hubbell 1975; Morse 1974, 1981; Ziv *et al.* 1993; Cresswell 1998), and may play an important role in the coexistence of trophically similar species (Carothers and Jaksic 1984). Direct interference may also be an

important process in the success of invading species and their detrimental effects on the native fauna (e.g. Human and Gordon 1996)

The European honeybee was first introduced into Tasmania in the early nineteenth century and feral colonies quickly spread to native vegetation (Ziegler 1993). Currently, most vegetation types throughout Tasmania support colonies of feral honeybees. Tasmania is also host to a commercial honey industry. The majority of honey production is derived from the Tasmanian leatherwood tree (*E. lucida*), a canopy co-dominant in cool temperate rainforest in the south and west of Tasmania (Ziegler 1993; Jarman *et al.* 1999). A large proportion of *E. lucida's* distribution lies within the Tasmanian Wilderness World Heritage Area.

The question of whether honeybees have a detrimental effect on native biota has generated substantial debate in recent years (e.g. Pyke 1990; Manning 1997). In Australia, a number of studies have examined aspects of exploitative competition between managed or hive honeybees on native insects (Pyke and Balzer 1985; Sugden and Pyke 1991; Paton 1996; Schwarz and Hurst 1997) and birds (Paton 1993, 1996). In Tasmania, Ettershank and Ettershank (1992; also Ettershank 1993) conducted a cursory examination of impacts of honeybees on insects associated with *E. lucida*, and concluded that honeybees have no exploitative impacts on native pollinators. The question of whether introduced honeybees impact on native pollinators through direct interference has received little attention.

This chapter reports on interference between honeybees and native insect visitors to *E. lucida* flowers.

Methods

Study sites and times

Data on insect visitors to flowers were gathered from fourteen rainforest sites between 1998 and 2000 (see *Study Sites* section for details of sites). Eight of the sites were situated in the vicinity of a commercial apiary site (number of hives ranged from 34-100), while the remaining six sites were >2 km from an apiary site. Sites >2 km from an apiary were outside the foraging range of the hive bees (see Chapter 10). The three Link Road sites were sampled in 1998 prior to the placement of commercial hives and again in 2000 with 100 hives at the two apiary sites. The Maydena site was sampled in early season (no hives), mid season (34 hives) and in

late season (102 hives). In total, I obtained video data from 19 separate sites/sessions, 9 in the presence of an apiary and 10 in the absence of an apiary.

Recording insects at flowers

I used Sony Handycam Video 8 cameras (models CCD-TR501E: 15X zoom and CCD-TR511E: 18x optical zoom) mounted on camera tripods to record insects visiting *E. lucida* flowers. Data were gathered on warm, clear days with a temperature maximum of >18° C. *E. lucida* tends to flower only when in full or partial sunlight, which in mature rainforest generally occurs in the canopy and adjacent to natural canopy gaps. For the video data, I used trees on the edges of roadsides and power-line clearings which flowered to near ground level, and used flowering branches on these trees from 1 m to 2.5 m above the ground.

I recorded insect visitors to flowers during three 1-hour sessions (1000, 1300 and 1600 hours) over a sampling day. Data were gathered over 3 to 5 days for each site. For each 1-hour session, a camera was placed 2-3 m from a flowering tree and trained on a set of 4-10 flowers for a 10-minute segment, after which the camera was moved to a new set of flowers on the same tree for a second 10-min segment. This was continued for 4 or 5 segments over the hour-long session. Different trees were used on different sessions and days. Data were recorded on Sony 8 mm (90-min) cassettes run on long-play mode (i.e. 180 minutes playing time per tape).

Tapes were analysed using a video recorder and TV monitor. For each 10-min segment, the number of flowers in clear view on the monitor was assessed, and only insect visits to these flowers recorded. For each segment, all visits to flowers were recorded. Flower visitors were classified as either native insects (classified to order or family level) or honeybees. I defined an 'interaction' as when two or more insects were present within a flower (regardless of whether the insects appeared disturbed) or where one insect approached another insect already in a flower. It is possible that interactions between insects also occurred away from flowers, or that an insect in a flower may have been disturbed by an approaching insect before the latter became visible on the video screen (viewing area *ca.* 30-50 cm^2) (*cf.* Morse 1977). However, my observations of foraging insects indicate that direct interference between floral visitors occurred only within flowers, or as the approaching insect came to within 1-2 cm of an occupied flower. I classified interactions as native-native, native-honeybee or honeybee-honeybee, and classed each interaction according to the result (no change, one disturbed, both disturbed). The taxon of the disturbed insect and

whether it was first- or second-comer was also recorded. An insect was taken to be 'disturbed' if its behaviour (feeding or approach) appeared to be altered by the vicinity of the second insect. Generally this was clear-cut, with the feeding insect completely vacating the flower, or the approaching insect clearly avoiding an occupied flower it was in the process of alighting on.

Results

Overall, I recorded 1193 10-minute video segments (199 hours total footage), and observed a total of 7737 flowers, giving a total of 1289.5 'flower-hours' of observation. During this time I observed a total of 1402 flower visits (including occasional repeat visits by the same insect), 552 (39.4%) of which were by honeybees and 850 (60.6%) by native insects. Flies were the most common native visitor (>70% of total native visitors), with beetles, native bees and native wasps, and occasional butterflies making up the remainder (see Chapter 3).

Table 6.1. Interactions between native insects and honeybees at *E. lucida* flowers, showing total number and results of interactions. Percentage of totals in brackets.

Interaction	Total	No Change	One Disturbed	Both Disturbed
Native/Native	14	7 (50.0)	4 (28.6)	3 (21.4)
Honeybee/Native	15	0 (0.0)	15 (100.0)[1]	0 (0.0)
Honeybee/Honeybee	6	0 (0.0)	0 (0.0)	6 (100.0)

[1]All native insects disturbed

Despite a total observation time of 199 hours (i.e. nearly twenty 10-hour days) and the large number of flower visits recorded, I observed only 35 interactions between insects at flowers (Table 6.1). All interactions were between just two insects. Fourteen were native-native interactions, the majority of which were between two flies or a fly and another insect, while 15 were honeybee-native interactions (Table 6.1). Seven of the native-native interactions (50.0%) resulted in no apparent disturbance of either visitor, four native-native interactions (28.6%) resulted in one insect being disturbed (all the first-comer), while three interactions led to both insects vacating the flower (Table 6.1). In contrast, all honeybee-native interactions resulted in disturbance of the native visitor (Table 6.1). Of these 15 honeybee-native interactions, the native insect was first-comer in 40% of cases. Significantly more honeybee-native interactions led to disturbance of a visitor than did native-native interactions (chi-squared test $X^2=9.7$, $P<0.025$, $df=3$). I observed only 6 interactions

between two honeybees, all of which resulted in the disturbance of both bees (Table 6.1).

The total flower-hours recorded at sites with and without an apiary were approximately equal (666.7 and 622.8 flower-hours, or 51.7% and 48.3% respectively). Assuming equal sampling effort at sites with and without an apiary, there were significantly more total insect visits to flowers at sites with an apiary than without an apiary (Table 6.2). There were significantly more honeybee visits recorded at sites with an apiary compared to sites without an apiary, while there were significantly more native visits recorded at sites without an apiary compared to sites with an apiary (Table 6.2).

Table 6.2. Total insect visits and the number of honeybee and native visits to *E. lucida* flowers with and without an apiary present at site. Results of Chi-squared tests are also shown.

Apiary	Total	Honeybees	Natives
Present	803	414	389
Absent	599	138	461
Chi-squared (df=1)	29.6***	136.0***	6.2**

**, P<0.025

***, P<0.001

The number of interactions as a proportion of total visits at sites with and without a apiary were not significantly different (Table 6.3). Both honeybee-honeybee and honeybee-native interactions tended to be more common near an apiary, although the differences were not significant (Table 6.3). Similarly, sites without an apiary tended to have a greater proportion of native-native interactions than sites with an apiary although the difference was not significant (Table 6.3).

Table 6.3. Total insect visits, total number of interactions and types of interaction between insect visitors to *E. lucida* flowers with and without an apiary present at site. Results of Chi-squared tests are also shown. Percentage of totals in brackets.

Apiary	Total	Interactions	Hb/Hb	Hb/Nat.	Nat./Nat.
Present	803	23 (2.9)	5 (22.7)	12 (52.2)	6(26.1)
Absent	599	12 (2.0)	1 (8.3)	3 (25.0)	8 (66.7)
Chi-squared (df)		1.1^{NS} (3)	1.07^{NS} (3)	2.3^{NS} (3)	5.3^{NS} (3)

^{NS}, P>0.1

Discussion

The frequency of interactions between insects at *E. lucida* flowers appears to be substantially lower than those recorded in other studies which have demonstrated direct interference between floral visitors. For example, in his examination of interactions between bumblebees and two species of syrphid fly on pasture rose (*Rosa carolina*), Morse (1981) recorded contacts between bumblebees at flowers every third minute of observation time. Similarly Hingston (1997) examined interference between honeybees and native halictid bees on *Carpobrotus rossii* in southern Tasmania, and observed as many as 63 interactions between honeybees and native bees in a single day.

The low rate of interactions between floral visitors to *E. lucida* is apparently due to the low numbers of both honeybees and native insects foraging on the flowers of a tree at any one time. Even in the vicinity of 100-hive apiaries with as a many as 50-60 000 bees per hive, moderately sized trees (with several hundreds to more than a thousand flowers) typically had < 5 honeybees working flowers at any one time. The number of native insects visiting flowers varied enormously between sites (range of 1.6-22.2 native visits/flower/10-hour day; Chapter 3). However, even at those sites where visits by natives were most frequent, individual *E. lucida* trees tended to have a small number of native insects foraging at any one time relative to the number of flowers available.

The potential for exploitation competition between introduced honeybees and native anthophilous insects and birds has been examined in a number of studies both in Australia (Pyke and Balzer 1985; Sudgen and Pyke 1991; Ettershank and Ettershank 1992; Ettershank 1993; Paton 1993, 1996; Schwarz and Hurst 1997) and elsewhere (Roubik 1978, 1983; Schaffer *et al.* 1978, 1983; Donovan 1980; Roubik *et al.* 1986). In contrast, the potential for interference competition between honeybees and native insects has received little attention. The results of the present study indicate that direct interference between honeybees and native visitors to *E. lucida* flowers does occur but at a very low rate. When honeybees and native visitors did interact at flowers, the result was invariably disturbance of the native visitor, while native insects were frequently observed feeding with another native at the same flower without apparent discomfort (Table 6.1). Hingston (1997) also found that when honeybees encountered native halictid bees at flowers of *C. rossii*, the result was almost invariably the disturbance of the native bee, while the native bees frequently foraged together on the same flower. Similarly, Morse (1981) found that bumblebees foraging on *R. carolina* were un-affected by the presence of native

syrphid flies at flowers while the native flies were almost always disturbed by bumblebees. Williams and Adam (1997) also found that feral honeybees in lowland subtropical rainforest disturbed native hylaeine bees wherever the two species occurred together at flowers. However, honeybees and native *Trigona* species were observed to forage together without apparent disturbance to the native bees (Williams and Adam 1997). Gross and Mackay (1998) examined the impacts of introduced honeybees on the pioneer shrub *Melastoma affine* in north Quseensland. Direct interactions between honeybees and native bees foraging on *M. affine* were recorded at flowers, with most honeybee-native bee interactions resulting in disturbance of the native bee (Gross and Mackay 1998).

It is not known whether the occasional interference observed in the present study between honeybees and native insects has a significant impact on the survival or reproductive success of the native species visiting *E. lucida* flowers. However, given the very low rate of interactions observed, significant competitive effects of honeybees via interference seem unlikely. Because of the relatively small number of insects foraging on *E. lucida* trees at any one time, there are generally very large numbers of unoccupied flowers available. Thus while the occasional disturbance of a native insect by a honeybee results in the native being driven off from the contested flower, it seems likely that the native could resume feeding on an alternative flower without major disruption to its foraging regime.

An alternative scenario is that an occasional displacement of native insects by honeybees results in the natives species avoiding entire trees being utilised by honeybees. Johnson and Hubbell (1975) described such a situation for two species of eusocial *Trigona* bees foraging on the shrub *Cassia biflora*, in which low, continual displacement of one species by the other led to the partitioning of entire shrubs between the two bees. Similarly, Morse (1977) described an example of displacement of small bumblebees by large bumblebees without any direct interaction or overt aggressive behaviour, with the small bumblees simply vacating a flower cluster when the larger species began to feed on the same branch. In the present case, however, the low number of honeybees foraging on individual *E. lucida* trees at any one time, and the very large numbers of flowers on individual trees (up to 4-5 thousand for mature trees; see Chapter 10), make it unlikely that foraging honeybees were defending entire trees from native visitors. I also observed no evidence that honeybees effected native insects until the former were 1-2 cm from a flower containing a native insect.

The low rate of interaction observed between honeybees and native insects applied to both sites distant from and sites in the near vicinity of a commercial apiary. Flowers of *E. lucida* do receive more visits from honeybees near

commercial apiaries (Table 6.2; see Chapter 8) although the number of honeybees foraging on *E. lucida* trees next to these apiaries is still remarkably low (Ettershank 1993; see Chapters 3 and 8). Furthermore, there were significantly more native insects at flowers at sites distant from commercial apiaries (Table 6.2). The introduction of commercial loads of hives into *E. lucida* forest therefore raises the number of honeybees at flowers, which could potentially lead to resource competition and a reduction in native visitors (see Chapter 8 and Appendix 2 for a more detailed discussion). However, direct interference between honeybees and natives was not greatly increased near hives and interference competition between hive bees and native visitors to *E. lucida* would appear to be minimal. However, the results of the present study were confined to flowers within 2.5 m of the ground. It is possible that honeybee visits are more frequent to flowers higher in the canopy, leading to an increased potential for interference competition compared to flowers near the base of the tree.

Chapter 7. Flowering phenology, nectar production, and potential honeybee impacts in Tasmania's cool temperate rainforest

Abstract

Five angiosperm species produced flowers with a nectar reward in a stand of cool temperate thamnic rainforest around Waratah in north-west Tasmania. Only *E. lucida* produced significant quantities of nectar sugar per hectare. The morning standing crop of nectar sugar in rainforest was very low (< 40 g sugar/ha) between April and December, but increased rapidly to > 1000 g sugar/hectare as *E. lucida* came into flower. The daily production of nectar in *E. lucida* flowers adjacent to an apiary was 1.08 mg sugar/flower/day, while daily consumption was 1.27 mg sugar/flower/day, with the difference made up by nghttime production. During peak flowering in February the forest was producing > 2000 g of sugar/ha/day. Honeybees and native dipterans were the principal insects taking nectar from *E. lucida* flowers. Given the rapid consumption of available nectar and the dearth of alternative floral resources in this type of forest, there is clearly a strong *prima facie* case for resource competition between hive honeybees and the native nectarivorous fauna associated with *E. lucida*.

Introduction

Tasmania's rainforests form the southern-most part of Australia's cool temperate rainforest assemblage (Read 1999). Unlike the northern mesothermal rainforests of this group, the southern cool temperate rainforests of Victoria and Tasmania are characterized by low species richness in their angiosperm component, with as little as half a dozen flowering plants within a stand (Jarman and Brown 1983; Jarman *et al.* 1999; Read 1999). Furthermore, among the numerically dominant canopy species, wind pollination tends to be the predominant pollen-dispersal system, with only Tasmanian leatherwood *E. lucida* and *Atherosperma moschatum* producing a nectar reward for the attraction of insect pollinators in Tasmania's cool temperate rainforest (Read 1999).

The nectar of *E. lucida* forms the basis of an established commercial honey industry in Tasmania, with leatherwood honey making up > 70% of the state's total honey production (Ziegler 1993). Despite the importance of *E. lucida* to commercial honey production, there is little information on the rates of nectar production in *E. lucida*-rich cool temperate rainforest, with the single study by Ettershank and Ettershank (1993) yielding minimal information on flowering and nectar production in this species.

This chapter describes the flowering phenology and nectar standing crop of thamnic cool temperate rainforest over a full calendar year, and provides data on the rates of production and consumption of nectar in *E. lucida* flowers in the vicinity of a commercial apiary. The patterns of flowering and nectar availability in cool temperate rainforest are discussed in terms of potential resource competition between hive honeybees and the native nectivorous insects visiting flowers.

Methods

Study Sites

The flowering phenology and nectar standing crop in thamnic cool temperate rainforest was studied at WAR1, WAR2, WAR3 and WAR4. Rates of nectar production and consumption and insect visits to flowers were studied at WAR1. See *Study Sites* section for details of sites. The forest at the study sites corresponded to thamnic cool temperate rainforest, community type T1.1 (Jarman *et al.* 1999), with an uneven canopy dominated by myrtle (*Nothofagus cunninghamii)*, leatherwood (*E. lucida*), sassafras (*A. moschatum*) and occasional trees of celery-top pine (*Phyllocladus asplenifolius*), over a dense sub-stratum of horizontal (*Anodopetalum biglandulosum*) with occasional native laurel (*Anopterus glandulosus*) and native plum (*Cenarrhenes nitida*). Occasional *Richea pandanifolia* were present at WAR4 but not at the other sites. Other species were occasionally present on roadsides and forest edges, including *Acacia melanoxylon*, *Cyathodes* sp., *Gahnia grandis*, *Trochocarpa* spp., *Telopea truncata*, *Leptospermum* sp., *Coprosma quadrifida*, *Tasmannia lanceolata*, *Gaultheria hispida* and *Phebalium squameum*.

Flowering phenology

Flowering phenology at all four sites was investigated between May 1997 and April 1998. During the initial visit in May 1997, I used a point-quadrat method (5 quadrat points per site, total n=20) to estimate the density of forest trees with a basal trunk diameter of > 10 cm (including re-sprouting *A. biglandulosum* stems) (Mueller-Dombois and Ellenberg 1974). During each subsequent monthly visit, I recorded the degree of flowering of forest trees. In the first instance, this involved walking through each site and recording whether or not a species was in flower. Where a forest tree was observed to be in flower, I estimated the density of flowers per tree for that month, as detailed below.

Flower density of *N. cunninghamii* was not scored, as the flowers of this wind-pollinated species are small and cryptic and do not produce nectar. For the two other principal canopy trees (*A. moschatum* and *E. lucida*), 10-15 trees per

site were selected each flowering month (total n=40-60 trees), with trees on the edge of the track or road being avoided to reduce the effect of the canopy gap on tree flowering. Using 8x40 binoculars, I approximated the shape of the exposed canopy of each tree (as a sphere, cone or cylinder), and estimated the dimensions (radius and height) required to calculate the canopy area. In addition, I scanned 3-4 points on the tree canopy and obtained a mean estimate of the number of flowers per square metre, allowing me to calculate the approximate number of flowers for each tree.

In the shrub *C. nitida*, flowers are carried on axillary spikes in clusters at and near the ends of branches. For a total of 20 flowering *C. nitida* distributed over the four sites, I counted the total number of branches bearing flower clusters, giving an average number of flowering branch-ends per shrub. For 10 of these shrubs, I counted the number of flowers per branch-end for 6-10 branches, giving an estimate of the average number of flowers per branch-end. In the shrub *A. glandulosus*, the large, showy flowers are carried on large terminal racemes, and can be counted individually. For 10 flowering *A. glandulosus* distributed over the four sites, I counted the total number of flowers per shrub. Flowering of *A. biglandulosum* was extremely patchy, with only sporadic branches observed in flower on the edge of the road in January. I obtained measures of nectar per flower for *A. biglandulosum*, but made no attempt to quantify the density of flowers per hectare for this species.

Morning standing crop of nectar

For each flowering species in the month in which flowering was first observed, I obtained a measure of the volume of liquid nectar (using 5μL and 20μL micropipettes) and the quantity of nectar sugar per flower using a hand-held refractometer. All measurements were conducted between 0800 and 0900 hrs. Because *E. lucida* tends to flower only when in full or partial sunlight, I used trees on the edges of the road-side clearing which flowered to near ground level, and used flowering branches on these trees from 1 m to 2.5 m above the ground. Flowers *of A. moschatum* and *A. biglandulosum* were taken from the lower branches of small trees on the road-side.

Flowers of *A. moschatum* and *C. nitida*, contained negligible volumes of liquid nectar. Total floral sugar was estimated in these species by rinsing a sample of flowers with two washes of 20μL of distilled water and measuring the sugar concentration of the wash fluid with a hand-help refractometer (see Appendix 1 for details of method). For *A. biglandulosum* and *E. lucida*, small volumes (generally <5μL maximum) of liquid nectar were extracted from flowers. Where sufficient liquid nectar was extracted to obtain a refractometer

reading (>4μL), a reading was obtained for that sample. This was followed by two 20μL rinses to extract any remaining sugar in the flower. Where <4μL of liquid nectar was extracted, the nectar was blown back into the flower and two 20μL rinses used to extract total floral sugar. For *A. glandulosus*, most flowers yielded sufficient liquid nectar to obtain a refractometer reading, after which the remaining sugar was extracted using two 20μL rinses with distilled water.

Nectar production and consumption in E. lucida *flowers*

I examined the daytime production and consumption of nectar by *E. lucida* flowers in the vicinity (within 400 m) of one of the commercial apiary sites over three days in late January, 1998. Total sugar per flower was measured in a sample of 10 flowers taken from three trees at 1000 hrs, and in a sample of 10 flowers from the same trees at 1800 hrs. In addition, I bagged one flowering branch of each sampling tree at dawn with fine nylon mesh (1 mm mesh-size) and measured total floral sugar in a sample 10-15 bagged flowers between 1600 and 1800 hrs.

Insects Visiting E. lucida *Flowers*

I used a Sony Handycam Video 8 camera (model no. CCD-TR501E; 15x variable zoom) mounted on a camera tripod (height=1.5 m) to record insects visiting flowers on trees adjacent to the road-side clearing. Insect visitors were recorded at 1000, 1300 and 1600 hrs over five days in February 1998 at the same apiary site used to investigate production and availability of nectar. For each session, a camera was placed 2-3 m from a flowering tree and trained on a set of 4-10 flowers for a 10-minute segment, after which the camera was moved to a new set of flowers on the same tree for a second 10-minute segment. This was continued for 4-5 segments over an hour-long session. Data were recorded on Sony 8 mm (90 minute) cassettes run on long-play mode (ie. 180 minutes playing time per tape).

Tapes were analyzed using a video recorder and TV monitor. For each 10-minute segment, the number of flowers in clear view on the monitor was assessed, and insect visits to these flowers recorded. For each segment, all floral visits were scored, with visitors classified as either honeybees or native insects (identified to family where possible). I calculated visitation rates as the number of visits per flower per 10-minute video segment. I then estimated the average number of visits a flower would be likely to receive over a 10-hour day by calculating the mean visitation rate for the 1000-, 1300 and 1600-hour sampling sessions combined, and multiplying this figure by 6 (=visits per hour) and by 10 (= visits per 10-hour day).

Results

A. biglandulosum was the most commonly recorded species (Table 7.1), reflecting the habit of this species to produce many secondary stems from the main trunk. Of the three canopy tree species, *N. cunninghamii* and *E. lucida* were similarly abundant, with slightly lower numbers of *A. moschatum* per hectare (Table 7.1). The two understorey shrubs were less abundant, but occurred in appreciable numbers scattered throughout the forest.

Table 7.1. Density (individuals/ha) of rainforest species

Species	Density
A. biglandulosum	524
N. cunninghamii	399
E. lucida	378
A. moschatum	273
A. glandulosus	21
C. nitida	42
Total	1637

There was no flowering activity recorded in the rainforest during the winter months (May-August), although *A. moschatum* was observed coming into bud in late August (Fig 7.1). During peak flowering in September, *A. moschatum* carried numerous flowers (up to one million flowers per hectare; Fig 7.1) with very small (<0.039 mg/flower) quantities of floral sugar per flower (Table 7.2). *C. nitida* flowered in November and December (Fig 7.1), with each flower containing extremely small amounts of floral sugar (<0.007 mg/flower; Table 7.2). In January, *A. glandulosus* and *A. biglandulosum* were observed in flower. *A. glandulosus* flowers contained appreciable quantities of a dilute nectar (Table 7.2), while *A. biglandulosum* flowers contained small (<0.4μL/flower) volumes of concentrated nectar (Table 7.2). Scattered trees of *E. lucida* commenced flowering in late December, with flowering in most trees underway by mid-late January (Fig 7.1). *E. lucida* flowers contained relatively small volumes of a dilute nectar in the early morning (Table 7.2). Flowering in *E. lucida* was heaviest in February (over two million flowers per hectare), with a reduced number of flowers still present on trees in March (Fig 7.1).

Combining the estimates of flower density (Fig 7.1) with the measurements of nectar levels in flowers before 0900 hrs (Table 7.2) gave an estimate of the total morning standing-crop of sugar present in the forest over a

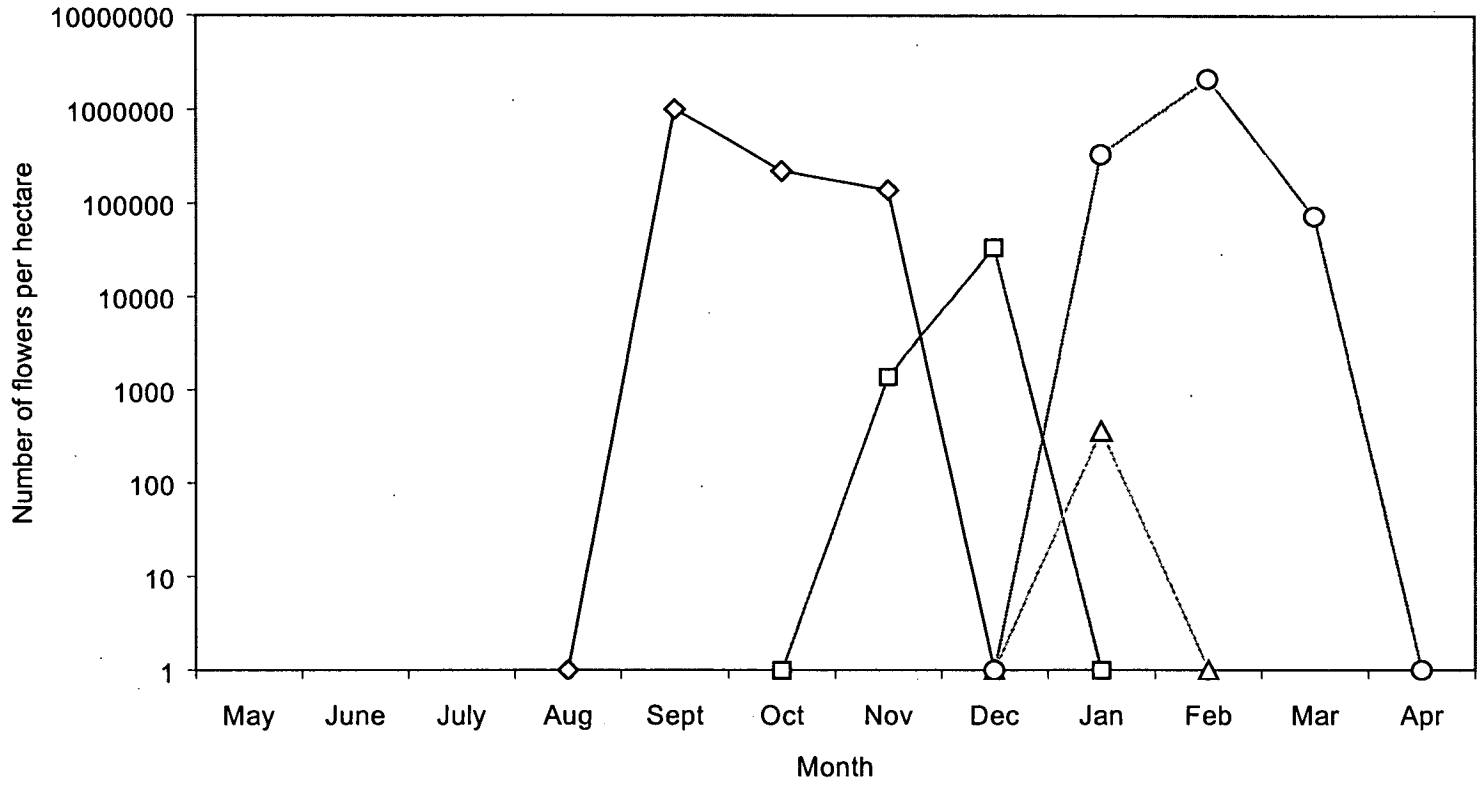

Fig. 7.1. Flowering phenology of rainforest species over a full year. Diamonds: *A. moschatum*, Squares :*C. nitida*, Triangles : *A. glandulo*sus, Circles: *E. lucida*,

full year (Fig 7.2). There was no sugar available in the forest in winter. Small quantities of sugar (*ca.* 40 g/ha) were produced in early spring during peak flowering in *A. moschatum*. Standing crop of sugar increased again in January with the onset of flowering of *E. lucida*. During peak flowering of *E. lucida* in February the standing crop of sugar in the forest exceeded 1000 g/ha (Fig 7.2).

The morning level of nectar sugar in *E. lucida* flowers in the vicinity of an apiary in late-January was 0.37±0.06 mg/flower (n=22; similar to the morning level of 0.48±0.08 mg/flower in December, see Table 7.2; and to the morning level of 0.52±0.06 mg/flower in February, see Table 8.1). Sugar levels in unbagged and bagged flowers in the late afternoon were 0.18±0.07 (n=25) and 1.45±0.18 mg/flower (n=40), respectively. Daily nectar sugar production was the difference between the morning and late-afternoon (bagged) nectar (=1.08 mg/flower/day), while daily nectar consumption was the difference between the late afternoon (bagged) and late-afternoon (unbagged) nectar (=1.27 mg/flower/day). More nectar was consumed than produced over a day, with the shortfall apparently made up by night-time production when most insects were inactive (see Chapter 1). A daily sugar production per flower of 1.08 mg translates into a daily production of >2kg of sugar per hectare per day by *E. lucida*-forest during peak flowering at WAR1 in February.

Table 7.2. Mean (±se) nectar volume (µL), nectar concentration (wt/wt), and total weight (mg) of sugar in rainforest flowers. All measurements taken between 0800-0900 hours.

Species	Month	Volume	Conc.	Weight	n
A. moschatum	Sep	negligable	-	0.039±0.005	35
C. nitida	Nov	negligable	-	0.007±0.002	15
A. glandulosus	Jan	6.36±0.79	13.49±1.76	1.12±0.24	11
A. biglandulosum	Jan	0.33±0.21	41.66±12.13[1]	0.77±0.14	15
E. lucida	Dec	0.66±0.22	19.83±1.20[2]	0.48±0.08	22

[1] n=3

[2] n=8

E. lucida flowers in the vicinity of the apiary received numerous visits from honeybees and native insects. The majority of native visitors were dipterans (primarily from familes Tachinidae, Calliphoridae, Muscidae, Tabanidae and Syrphidae), with small numbers of beetles and native bees also visiting flowers (see Chapter 3). The majority of honeybees were foraging for

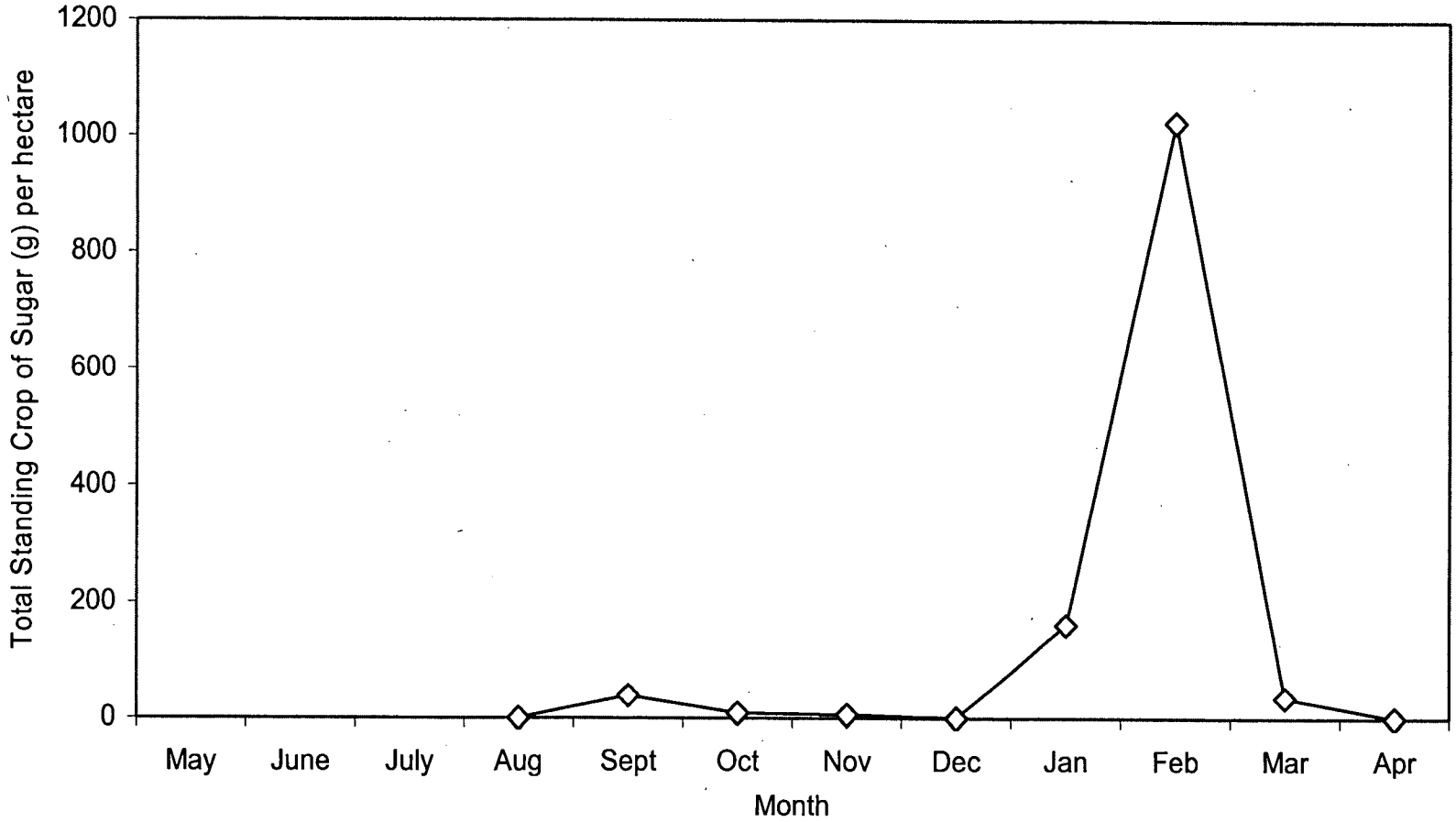

Fig. 7.2. Standing crop of sugar in the rainforest over a full year at the Waratah sites.

nectar although occasional pollen-collecting bees were observed on flowers. All non-syrphid flies were nectar-feeders, while syrphids collected both nectar and pollen, often during a single visit. Visit rates for honeybees and total native insects ranged between 0.14 – 0.24 visits per flower per 10-minutes (Fig 7.3). Honeybees tended to be more common than native insects throughout the day (see Chapter 8). Conversion of the data in Fig 7.3 to numbers of visitors over a 10-hour day gave an estimate of approximately 22 visitors to a flower over a day, with honeybees making up 57.7% of total visitors to flowers.

Discussion

Cool temperate rainforest tends to be floristically and structurally simple compared to rainforests in more equable climates at lower latitudes (Read 1999). Species richness in these cool temperate forests is confined to bryophytes and lichens, while the angiosperm component is characterized by only a handful of tree species and a relatively limited variety of understorey shrubs (Jarman *et al.* 1999). At the sites studied in north-west Tasmania, less than 10 angiosperm species were common within the rainforest proper, although a range of shrubby species occurred on road-sides and forest margins. Furthermore, from the viewpoint of nectar resource, the rainforest was even more simplistic, with only five of the common species producing floral displays with a nectar reward (*A. moschatum*, *E. lucida*, *A. biglandulosum*, *A. glandulosus* and *C. nitida*). Of the two numerically dominant canopy trees, only the flowers of *E. lucida* produced a significant quantity of nectar sugar/hectare.

Individual flowers of *E. lucida* contained relatively small volumes of a dilute nectar in the early morning. Although the nectar exudate of *E. lucida* is relatively dilute, the secreted nectar is rapidly concentrated to >70% wt/wt through evaporative water loss during the heat of the day (see Chapters 1 and 3). This concentrated nectar is highly attractive to insects. Nectar production in *E. lucida* continues throughout the day and flowers protected from insects rapidly accumulate nectar sugar (see Chapter 1). However, the rate of sugar production recorded at the four Waratah sites in January 1998 (*ca.* 1 mg per flower per day) is relatively modest compared to other species used in the production of honey (see also Table 9.1 for full range of nectar production rates in *E. lucida* flowers). For example, daily nectar production in species of *Banksia* can exceed 250 mg per inflorescence per day (McFarland 1985, 1986), while Paton (1986) reported nectar production in species of eucalytps of up to 25 mg per flower per day.

Despite a relatively modest per flower secretion rate, the per hectare production of nectar sugar in *E. lucida*-forest (> 2000 g/ha/day) compares favourably with nectar production in *Banksia*-dominated forests and heaths in

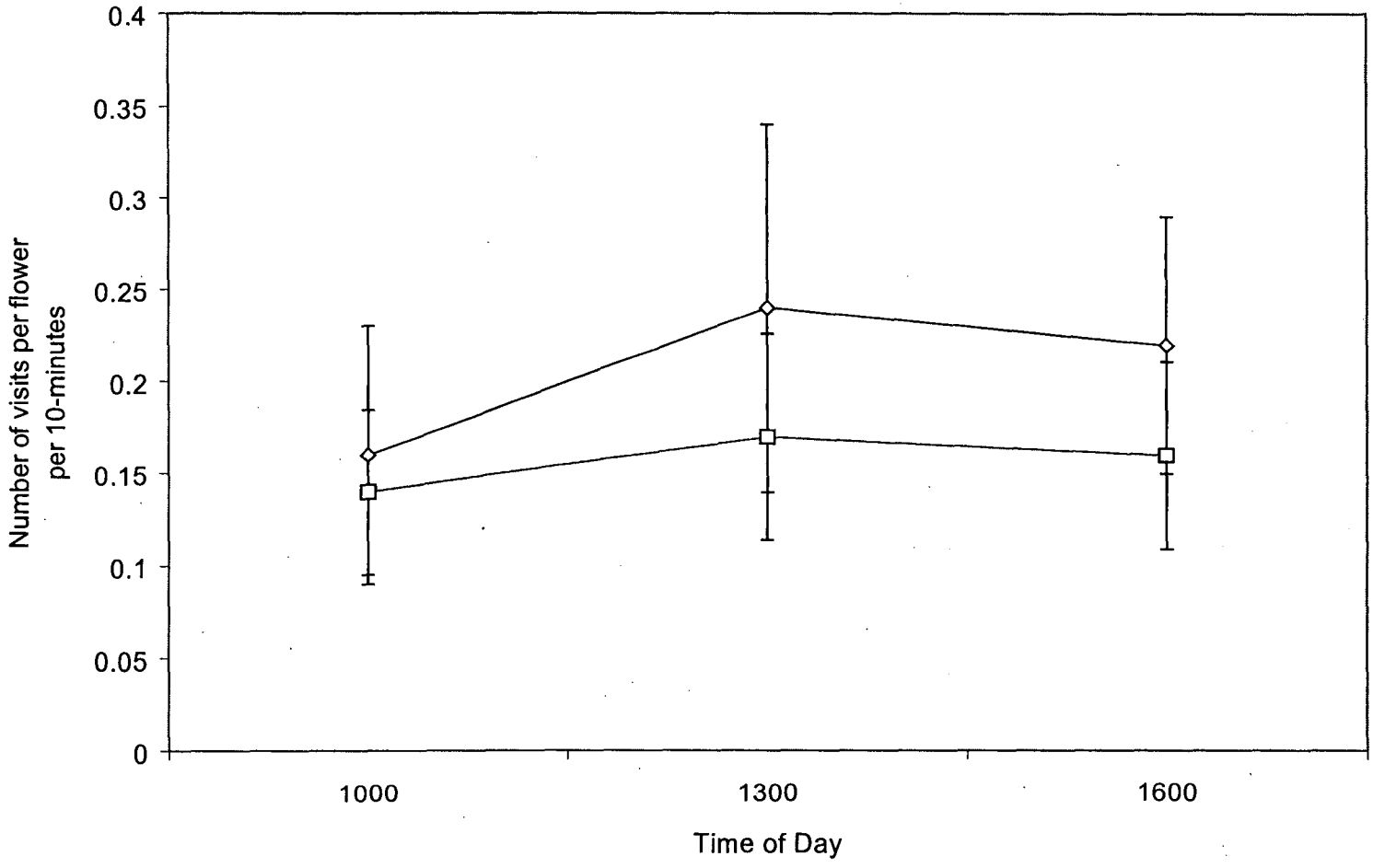

Fig. 7.3. Visit rates for honeybees (diamonds) and native insects (squares) at *E. lucida* flowers. n=18-24 for all points.

New England National Park (up to 1125 g sugar/ha/day in late winter: McFarland 1986) and in South Australia (up to 1000 g sugar/ha/day: Paton 1996), and is considerably greater than the nectar production in *Banksia*-dominated heaths near Sydney (around 100 g sugar/ha/day: Pyke and Recher 1986), in woodland areas adjacent to these heathlands (up to 225 g sugar/ha/day: Pyke 1985), and in Jarrah forests in Western Australia (150 g sugar/ha/day: Collins and Newland 1986). However, nectar production in eucalypt forests during heavy flowering of *Eucalytus* spp. may be substantially greater than in both *Banksia* heaths and leatherwood forest (e.g. up to 20 kg/ha/day in ironbark *E. sideroxylon* forests; Paton 1996), although eucalypt flowering tends to be both sporadic and unpredictable compared to *E. lucida* (Manning 1997).

Flowers of *E. lucida* received numerous visits (>20) over a day, with honeybees and native dipterans the principal visitors consuming nectar. Insects consumed 100% of the nectar produced during daylight hours and close to 50% of the morning standing crop, with overnight production replenishing the consumed nectar and ensuring flowers contain small amounts of nectar when insect activity resumes the following day. Honeybees were the most frequent visitor to flowers, making up 57% of total visits, and are likely to be consuming an even greater proportion of total nectar given their large size and efficient foraging behaviour at flowers (Paton 1996). Bond and Brown (1979) estimated that honeybees consume 13-20% of nectar produced by *E. incrassata*, while Collins *et al.* (1984) estimated that honeybees consumed 34% of the nectar produced by *Calothamnus quadrifidus*. Paton (1985) studied nectar consumption in a range of bird-pollinated heathland species and estimated percentage nectar consumption by honeybees ranged from 34-52%. Similarly, in his review of honeybee impacts, Paton (1996) concluded that honeybees typically account for greater than 80% of total visitors to insect-pollinated flowers, and that their share of floral resources would be at least this high.

Potential impacts of honeybees

The leatherwood-honey industry is concentrated into the 1-2 months of *E. lucida* flowering, with commercial operators moving their hives into rainforest sites with the onset of leatherwood 'honeyflow' in late December and January. Most leatherwood apiary sites occur in the north-west, west and the south of the state, while leatherwood forest in the south-west is inaccessible to commercial operators. Of the state's total leatherwood apiary sites (n=444), a significant number (n=63 or 14.2%) are located within or adjacent to the Tasmanian Wilderness World Heritage Area, although <10% of the total leatherwood forest

resource within these reserves is currently accessible for commercial use (Ziegler 1993).

The potential impacts of commercial honeybees on the native flora and fauna of Australia has generated substantial debate in recent years (Pyke 1990; Manning1997; New 1997). However, despite a recent increase in experimental studies (reviewed in Paton 1996), there is no clear consensus as to the likelihood and significance of commercial honeybee impacts on native fauna and flora. Principal reasons for this are the highly transient and un-predictable nature of most flowering resources and their associated industries (Manning 1997), the floral complexity of the natural systems into which commercial loads of honeybees are typically introduced, and the tendency for impacts to be highly site and season specific (Paton 1996).

The potential impacts of commercial honeybees on native fauna and flora clearly has substantial relevance in the Tasmania context, with a significant number of leatherwood apiary sites situated within reserves. Furthermore, the relative floristic simplicity of the cool temperate rainforest system makes it particularly suitable for investigating potential honeybee impacts. Leatherwood honey is derived almost exclusively from *E. lucida* nectar due to the absence of other significant nectar sources within the rainforest, making it effectively a mono-specific system. This is in contrast to the majority of apiary industries on mainland Australia in which honey is drawn from a variety of heathland and eucalypt species. Investigating honeybee impacts in Tasmania's mono-specific *E. lucida* system would greatly simplify key experimental aspects of this type of research, particularly the monitoring of resource (nectar and pollen) levels, and quantifying native insect activity at flowers without the confounding effects of shifts between abundant, alternative resource species. Data on nectar production and consumption in *E. lucida* flowers in the vicinity of an apiary site suggest that the entire daily nectar output by *E. lucida* flowers may be consumed by insects, with honeybees making up the majority of flower visitors in the vicinity of an apiary. Under such conditions of limited nectar availability, few alternative nectar sources and high honeybee activity at flowers, resource competition between hive bees and native insects in *E. lucida* forest is clearly a strong possibility.

Chapter 8. Impacts of hive honeybees on *E. lucida*: I. Honeybee activity, nectar levels and native anthophilous insects

Abstract

I examined the impacts of hive honeybees on native insect visitors to flowers of *E. lucida* by comparing sites in the vicinity (within 400 m) of a commercial apiary with control sites > 2 km from the nearest apiary. The level of honeybee activity at *E. lucida* flowers was significantly elevated near apiaries, and this increase in honeybee foraging pressure resulted in a significant depression in available nectar sugar in flowers. In the vicinity of an apiary, more nectar was consumed than produced over the day (*ca* 125% of total diurnal production consumed), while at control sites only *ca* 25% of total diurnal production was consumed by insects. Despite this reduction in available nectar, there was no difference between apiary and control sites in either visitation rates by native insects (mainly large dipterans) to flowers, or in the general abundance of native insects caught on sticky traps. The absence of any competitive effect of hive honeybees on native insects is attributed to an apparent superabundance of nectar produced by *E. lucida*. However, hive honeybees did appear to be competitively excluding feral honeybees from the vicinity of apiary sites.

Introduction

The potential impacts of the introduced honeybee on the native flora and fauna of Australia has generated substantial debate in recent years (see Paton 1996 for a recent review). The debate has tended to polarise around two opposing camps: on the one hand, the position that honeybees are a feral species, and therefore inherently detrimental for natural systems; on the other (typically an industry position), that honey production is a sustainable, environmentally friendly industry with no negative impacts on native biota (Pyke 1990; Manning 1997). Caught in the middle are land managers, who must make difficult decisions regarding the acceptability and/or extent of commercial apiculture in areas designated for the conservation of natural values (Pyke 1990; New 1997).

Honeybees may impact on the native pollinator fauna through interference or resource competition, and on the forage species through effects on pollen flow and fruit and seed set (Pyke 1990). Honeybee impacts may be due either to managed hives moved into native vegetation during conditions of honeyflow, as well as to feral colonies which reside in the bush year-round (Paton 1996). A number of studies have examined impacts of hive bees in Australia (Pyke and Balzar 1985; Sudgen and Pyke 1991; Paton 1993, 1996, 1997, 1999; Schwarz and Hurst 1997; Gross and McKay 1998) and overseas (e.g. Roubik 1978;

Murphy and Robertson 2000), while the potential impacts of feral bees has received scant attention (Paton 1996; New 1997).

Honeybees were first introduced into Tasmania in the early nineteenth century and feral colonies quickly became established in native vegetation (Ziegler 1993). Feral colonies of honeybees are currently found in all vegetation types throughout the state with the exception of high altitudes (Ziegler 1993). Tasmania is also host to a commercial honey industry, with > 70% of total honey production made up of leatherwood honey derived from the nectar of *E. lucida* (Ziegler 1993). *E. lucida* occurs as a canopy co-dominant in thamnic cool temperate rainforest in western Tasmania in areas of high rainfall and low fire frequency (Jarman *et al.* 1999). Leatherwood honey is harvested from native cool temperate rainforest in the north-west and west of the state, with hives moved into the bush in early-mid summer with the onset *of E. lucida* flowering. A significant portion of the rainforest employed for leatherwood honey production is located within reserves, and the potential negative impacts of both managed and feral bees is an ongoing issue for Tasmania's conservation land managers (Tasmanian Wilderness World Heritage Area Management Plan 1999).

To date, only a single study (Ettershank and Ettershank 1992; also Ettershank 1993) has investigated the potential impacts of honeybees on the native insect fauna associated with *E. lucida*. Ettershank and Ettershank (1992) concluded that honeybees had no effect on the abundance of native insects visiting *E. lucida* flowers. However their study was constrained by a number of methodological limitations, particularly control sites located less than 1 km from an apiary (i.e. well within the foraging range of hive-bees; see Chapter 10), their inability to sample the highly concentrated nectar from flowers after mid-morning, and their use of binoculars to count native insects from a distance of 10-20 m from a tree.

The present study aimed to more fully examine the potential impacts of commercial honeybee apiaries on the pollination ecology and native insect fauna of *E. lucida* flowers. In this chapter I examine the impacts of hives on honeybee numbers, nectar levels, and the abundance and activity of native insects at flowers. The impacts of hives on pollen levels and fruit and seed set in *E. lucida* are considered in Chapter 9.

Methods

Experimental design

I investigated the impacts of hive honeybees using two experimental approaches. First, I compared apiary sites (sites within 400 m of an active commercial apiary) with control sites (sites located a minimum of 2.0 km distant from the nearest apiary, assuming a foraging range for honeybees under conditions of honeyflow of 1-2 km; see Chapter 10). All apiary sites had a long history of apiary use, while none of the control sites had been employed for commercial honey production. I studied a total of 7 apiary and 6 control sites. Second, I employed a before-and-after-control design at three of the sites along the Western Explorer Link Road in north-west Tasmania. These sites had been opened up by a road built in 1997, had no previous history of apiary use and were at least 5 km from the nearest existing apiary site. The three sites were sampled in a control year (1998) when all sites were free of apiaries. Two of the sites were then taken over by a commercial apiarist and received 100 hives in 1999 and 2000 while the third site remained free of hives. I visited the sites in 1999 but did not sample due to bad weather curtailing the flowering period. I revisited the sites in 2000 and re-sampled the two apiary sites and the control site.

Study sites

The impacts of hive bees were studied at thirteen sites (WAR1-6, QT1-4 and LR1-3) from three locations in north-west and west Tasmania. See *Study Sites* section for details of sites. WAR1, WAR2, WAR4 and WAR5 were studied in February 1998, while WAR3 and WAR6 were studied in February 2000. QT1 and QT3 were studied in January 1999, and QT2 and QT4 were studied in January 2000. The Link Road sites were studied initially in late January/February 1998 when all sites were free of apiaries, and again in late-January 2000 when two of the sites (LR1 and LR2) had 100 hives, while the third site (LR3) remained free of hives.

Data collection

I collected data from pairs of apiary and control sites on the same days in order to reduce effects of variation between days on nectar production, insect abundance and insect activity at flowers. This allowed a more robust comparison between apiary and control sites. All data were gathered on warm days with a temperature maximum of > 18°C. Data on honeybee activity, nectar levels and insect visits to flowers were collected at three times of day (between 0900-1100 hours, 1200-1400 hours and 1500-1700 hours; hereafter the 1000-,

1300- and 1600-hour sampling sessions) on 3-5 days for each site. For the Waratah and Queenstown sites, data were gathered either by two people working simultaneously, one at an apiary and one at a control site (WAR1, WAR2, WAR4 and WAR5 and QT1 and 3), or by a single person working at an apiary site for one hour and then moving to a non-apiary site for the next hour of a sampling session (WAR3 and WAR6 and QT2 and QT4). The order of the apiary/control sites was alternated on different days. For the three Link Road sites, one person worked at one site while another person gathered data from the two other sites during each two-hour sampling session. The order in which the Link Road sites were visited was alternated on different days. Data on insect abundance using sticky traps were also gathered in pairs of apiary and control sites (see below).

Honeybee activity scans

I used a scan-technique to obtain an index of honeybee activity at *E. lucida* flowers. For each sampling session, 100 flowers from five different trees were scanned from the road-side using 8x40 binoculars, and the number of honeybees encountered at flowers recorded. Flowers were scanned at a rate of approximately 100 flowers per minute.

Available nectar sugar in E. lucida *flowers*

E. lucida trees tend to flower only when in full or partial sunlight, which in mature rainforest generally occurs in the canopy and adjacent to natural canopy gaps. For these experiments, I used trees on the edges of the roadside which flowered to near ground level, and used flowering branches on these trees from 1 m to 2.5 m above the ground. For each sampling session, I measured the quantity of floral sugar in a sample of 10 *E. lucida* flowers collected from 2 or 3 trees. Floral sugar was washed from flowers using two rinses with 20 μL of distilled water and the concentration of the wash fluid measured using a hand-held refractometer (see Appendix 1 for details of wash method). The sample of flowers was collected from different trees on the three sampling sessions during a day, and where possible from different sets of trees on different days.

Daily nectar production and consumption

I measured diurnal production and consumption of nectar at all sites, with the exception of WAR2 and WAR 4 and the three Link Road sites in 1998. On two of the sampling days at each site I bagged a single flowering branch on two trees between 0900 and 1000 hours with fine nylon mesh (1 mm mesh-size) and measured total floral sugar in a sample of 10-15 of these bagged flowers

between 1700 and 1800 hrs on the same day. Daytime production was taken as the difference between the late-afternoon bagged and the morning (1000 hour session) nectar levels, while nectar consumption was taken as the difference between the late-afternoon bagged and afternoon (1600 hour session) un-bagged nectar levels.

Insect visits to flowers

I used two Sony Handycam Video 8 cameras (model nos. CCD-TR501E: 15x optical zoom and CCD-TR511E: 18x optical zoom) mounted on camera tripods (height=1.5 m) to record diurnal insect visitors to flowers during the 1000-, 1300- and 1600-hour sampling sessions. Video data were gathered from the same trees used for nectar sampling. For each sampling session, a camera was placed 2-3 m from a flowering tree, trained on a set of 4-10 *E. lucida* flowers and run for a 10-minute segment, after which the camera was moved to a new set of flowers on the same tree for a second 10-minute segment. This was continued for 4-5 segments over approximately one hour. Data from a single sampling session usually came from a single tree. Different trees were used on the three sampling sessions during a single day, and where possible different sets of trees were used on different days. Data were recorded on Sony 8 mm (90 minute) cassettes run on long-play mode (i.e. 180 minutes playing time per tape).

Tapes were later analyzed using a video recorder and TV monitor. For each 10-minute segment, the number of flowers in clear view on the monitor was assessed, and only insect visits to these flowers recorded. For each 10-minute segment, I scored all feeding visits (visit duration > 1 second with obvious feeding behaviour), with visitors recorded as honeybees, large (≥ 5 mm) or small diptera (< 5 mm), large coleoptera (≥ 3 mm), native hymenoptera, or lepidoptera. Honeybees were further divided into those with a golden-coloured abdomen (typical of Italianate-race hive bees, *A. mellifera ligustica*) and those which were dull-coloured or black (typical of feral honeybees). Honeybee visits were also classified as either nectar-collecting (bee taking nectar with no obvious raking of pollen) or pollen-collecting (where the bee clearly raked pollen with its legs). I calculated visitation rates as the number of visits per flower per 10-minute video segment.

Invertebrate sampling

I used sticky-traps to sample the abundance of invertebrates in the rainforest. Sticky-traps were constructed from round food-container lids (translucent polypropylene, 12 cm diameter). Each lid was smeared on one side with a thin layer of Stickem™ and either attached by two nails near the top of a 1.5 m garden stake or attached to a loop of wire (30 cm length) tied to a flowering branch. Twelve traps were located among the lower foliage of *E. lucida* trees at approximated 25 m intervals at each site. Twelve traps were set for three days at all sites, while a second set of 12 traps was set for another 3 days after an interval of between 7-10 days at WAR1, 2, 4 and 5 and at LR1-3 in 1998. Sticky traps were set on the same days at WAR1, WAR2, WAR4 and WAR5 (February 1998), WAR3 and WAR5 (February 2000), all QT sites (January 2000), and at all LR sites (January 1998 and January 2000). After the three days sticky traps were frozen and analysed at a later date. All larger insects (> 1 mm) caught on the traps were scored under 40x using a binocular microscope as large (≥ 5 mm) or small dipterans (< 5 mm), coleopterans, hymenopterans, and spiders.

Statistical analysis

I tested for effects of hive honeybees in two ways. First, I compared treatment sites in the vicinity of an apiary (7 sites) with control sites > 2 km from an apiary (6 sites). For the honeybee-scan and nectar data, I used a hierarchical ANOVA with location and day as random factors and time of day and apiary as fixed factors. Data were log transformed to improve normality and to reduce heteroscedacity. However, the log-transformed nectar data still showed appreciable departures from homogeneity of variance due to some sites having much greater variation than others, and results are treated conservatively.

The raw video data included numerous zero values and was not amenable to analysis by ANOVA. Therefore I also combined the video data for different sampling days and then used a two-way ANOVA on the mean values for each site to test for an effect of apiary and time of day on mean visitation rates for honeybees, large dipterans, and for all native insects combined. For the sticky-trap data, I used a students t-test to test for an effect of apiary on the mean number of insects per trap.

Second, I examined the effect of introducing commercial honeybee hives at the Link Road sites using a before-and-after-control design. Due to the lack of replication in this design, I was not able to test for an effect of apiary directly. Therefore I used a three-way ANOVA to test for an effect of year, site and time of day on the number of honeybees per 500 flowers and on nectar sugar per flower. Because the video data included numerous zeros I confined my analysis to within-site comparisons between years on pooled video data. I pooled the video data for different days and times of day and used a simple students t-test to test for differences between years in the visitation rates by honeybees, large dipterans and total native insects. Similarly, I used a students t-test to test for within-site differences between years in the number of large dipterans and total insect per sticky-trap.

Mean data are presented ± standard errors.

Results

Honeybees

Significantly more honeybees were recorded in the 500-flower scans at the apiary sites compared to control sites ($F_{1,97}$=53.82, P<0.001) while there was no effect of time of day ($F_{2,97}$=2.34, P>0.1) and no interaction between factors ($F_{2,97}$=0.16, P>0.5) (Fig. 8.1). The mean number of honeybees recorded per 500 flowers ranged from < 0.75 (WAR5) to > 10 (LR2.2000 (Fig. 8.1). The mean number of honeybees recorded per 500 flowers was 5.49±0.52 (n=80) and 1.15±0.18 (n=72) for apiary and control sites, respectively.

Similarly, honeybee visitation rates from the video footage were significantly higher at apiary sites compared to control sites ($F_{1,33}$=14.92, P<0.001), while there was no effect of time of day ($F_{2,33}$=0.13, P>0.5) and no interaction between factors ($F_{2,33}$=0.28, P>0.5) (Fig. 8.2). The mean visitation rate by honeybees varied by greater than an order of magnitude between sites, ranging from 0.01 visits/flower/10-minutes (QT3) to 0.21 visits/flower/10-minutes (WAR1) (Fig. 8.2). Mean honeybee visitation rates were 0.11±0.01 (n=399) visits/flower/10-minutes at apiary sites and 0.04±0.01 (n=337) visits/flower/10-minutes at control sites.

The effect of an apiary on the number of honeybees recorded per 500 flowers was clearly apparent at the Link Road sites (Fig. 8.3). There was no effect of site ($F_{2,66}$=1.71, P>0.1) or time of day ($F_{2,66}$=1.08, P>0.1) but a significant effect of year ($F_{1,66}$=50.82, P<0.001) and a significant interaction

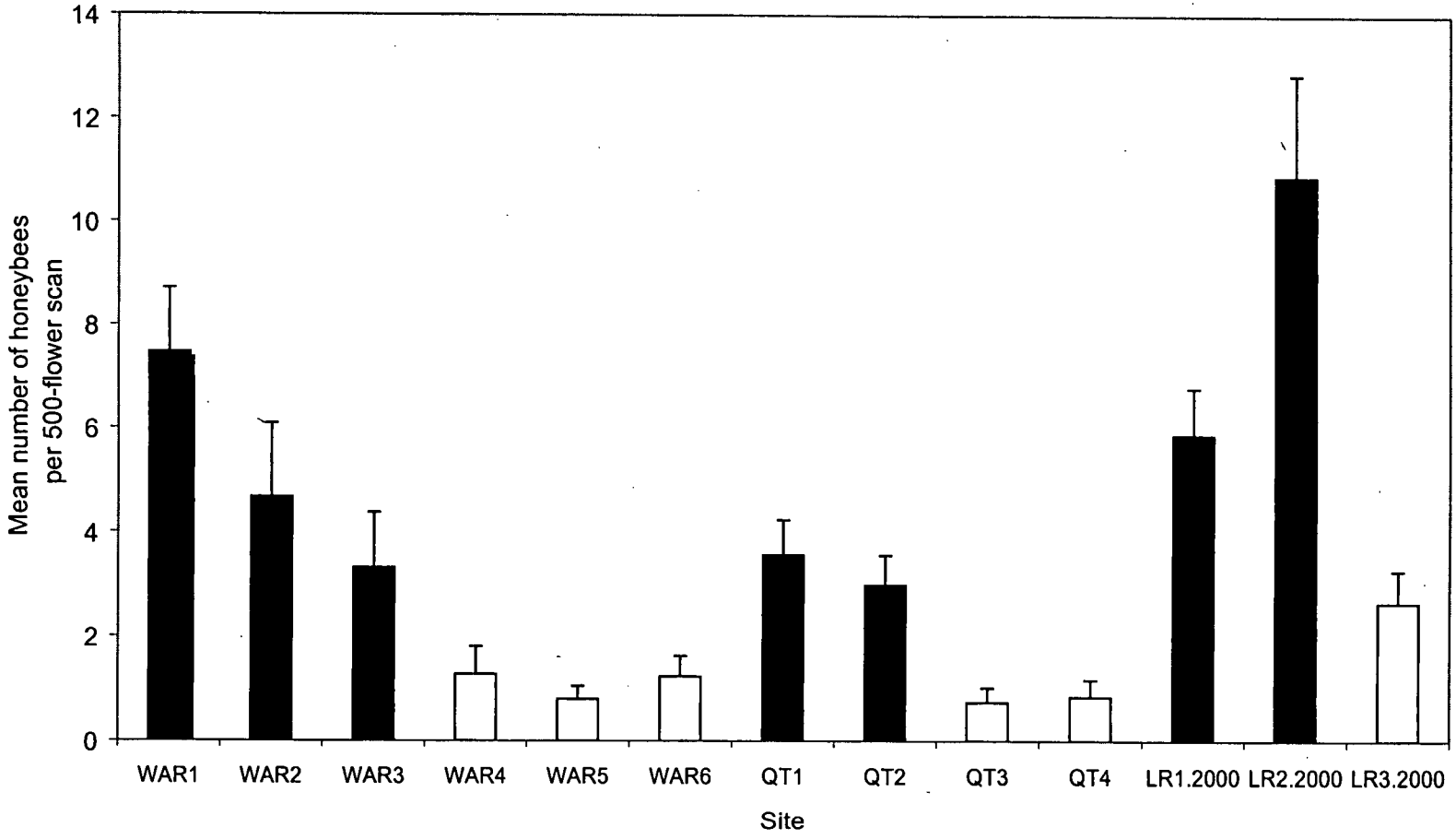

Fig. 8.1. Mean number of honeybees recorded during scans of 500 *E. lucida* flowers at apiary and control sites. Data for different days and times of day combined. Filled bars are apiary sites, open bars are control sites. n=8-15 scans for all sites. Error bars are standard errors.

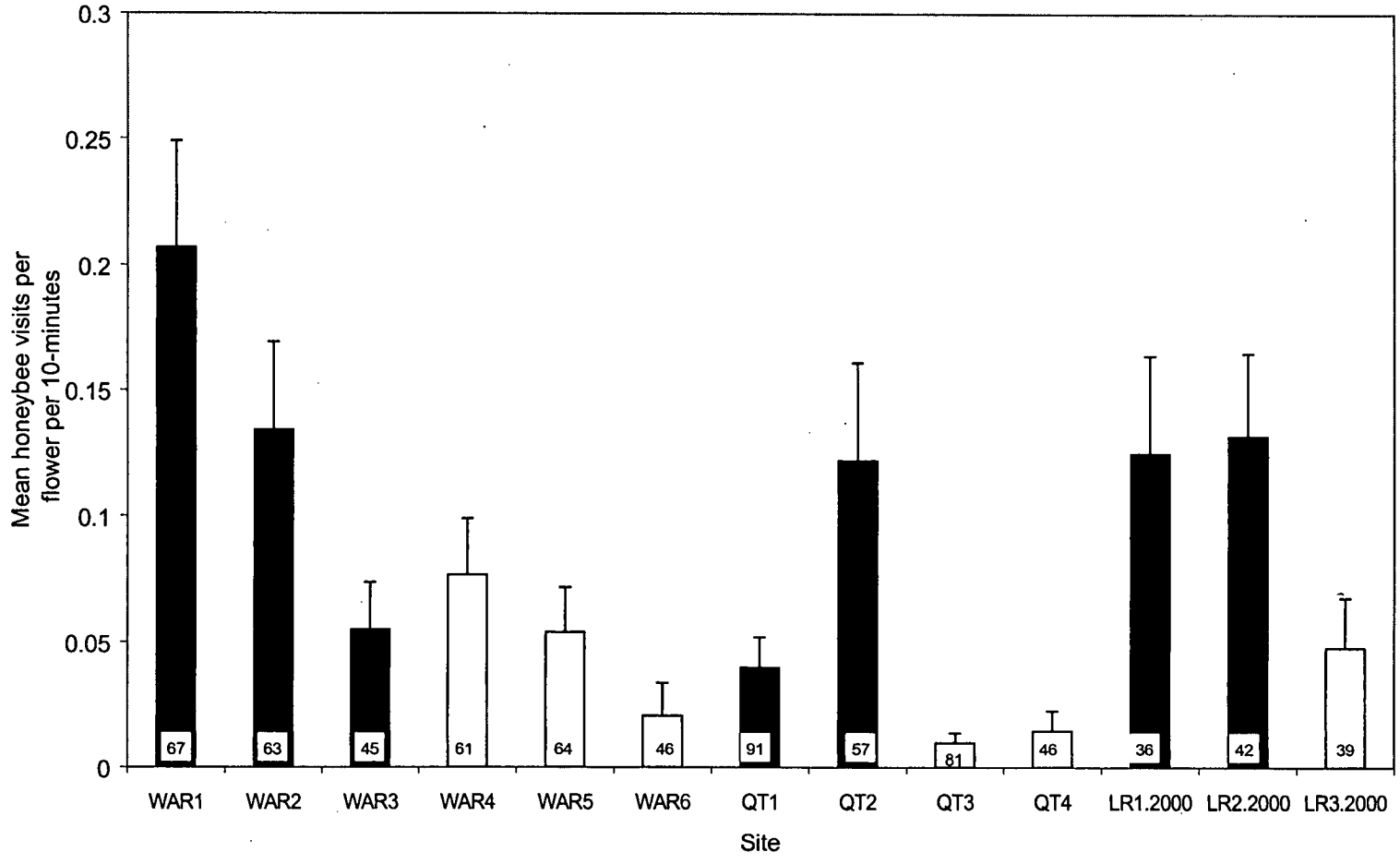

Fig. 8.2. Mean number of honeybee visits per *E. lucida* flower per 10-minutes from video footage at apiary and control sites. Data for different days and times of day combined. Filled bars are apiary sites, open bars are control sites. Sample sizes given at bottom of bars. Error bars are standard errors.

between site and year ($F_{2,66}$=12.72, P<0.001). The number of honeybees per 500 flowers increased by a factor of 3.3 and 11.5 at LR1 and LR2, respectively, in the second year after the introduction of 100 hives to each site (Fig. 8.3). In contrast, at the control site LR3, honeybees recorded per 500 flowers increased by a factor of only 1.1 in the second (Fig. 8.3). The effect of an apiary on visitation rates by honeybees from the video footage was also clearly apparent at the Link Road sites (Fig. 8.4). Honeybee visitation rates increased by a factor of 3.6 (t_{109}=2.81, P<0.01) and 26.4 (t_{115}=5.00, P<0.001) at LR1 and LR2, respectively, in the second year after the introduction of 100 hives. In contrast, honeybee visitation rates increased by a factor of only 1.4 at the control site LR3 in the second year (t_{111}=1.20, P>0.2) (Fig. 8.4).

Nectar and pollen collecting by honeybees

I observed a total of 403 visits by honeybees on videotape, 368 (91.3%) of which involved only nectar-collecting behaviour, 33 (8.2%) only pollen-raking behaviour, and 2 (0.5%) where both nectar-collecting and pollen-raking were observed during the same visit. Although relatively few honeybee visits were associated with active pollen collection, the constant contact between anthers and honeybees as they probed for nectar suggests that substantial amounts of pollen are also picked up passively during nectar visits.

Nectar

Nectar levels in un-bagged flowers varied substantially between sites and over the day (Table 8.1). In general however, nectar levels at apiary sites tended to either decline over the day or to remain at low levels throughout the day. In contrast, nectar levels at control sites tended to increase between the morning and afternoon sessions (Table 8.1). At all but one of the control sites, nectar sugar exceeded 0.7 mg of sugar per flower in the afternoon sampling session, with afternoon nectar levels at three of the control sites (WAR6, QT3 and QT4) exceeding 2 mg per flower. In contrast, nectar sugar in the afternoon was < 0.4 mg of sugar per flower at 5 of the 8 apiary sites (Table 8.1). The maximum quantity of sugar recorded in an open flower was 33.4 mg during the afternoon at QT3.

The effect of apiary on nectar levels in un-bagged flowers was highly significant ($F_{1,1623}$=7.99, P<0.005). There was no effect of time of day ($F_{2,1623}$=0.43, P>0.5) but a significant interaction between apiary and time of day on nectar levels ($F_{2,1623}$=12.14, P<0.001). The daytime production and

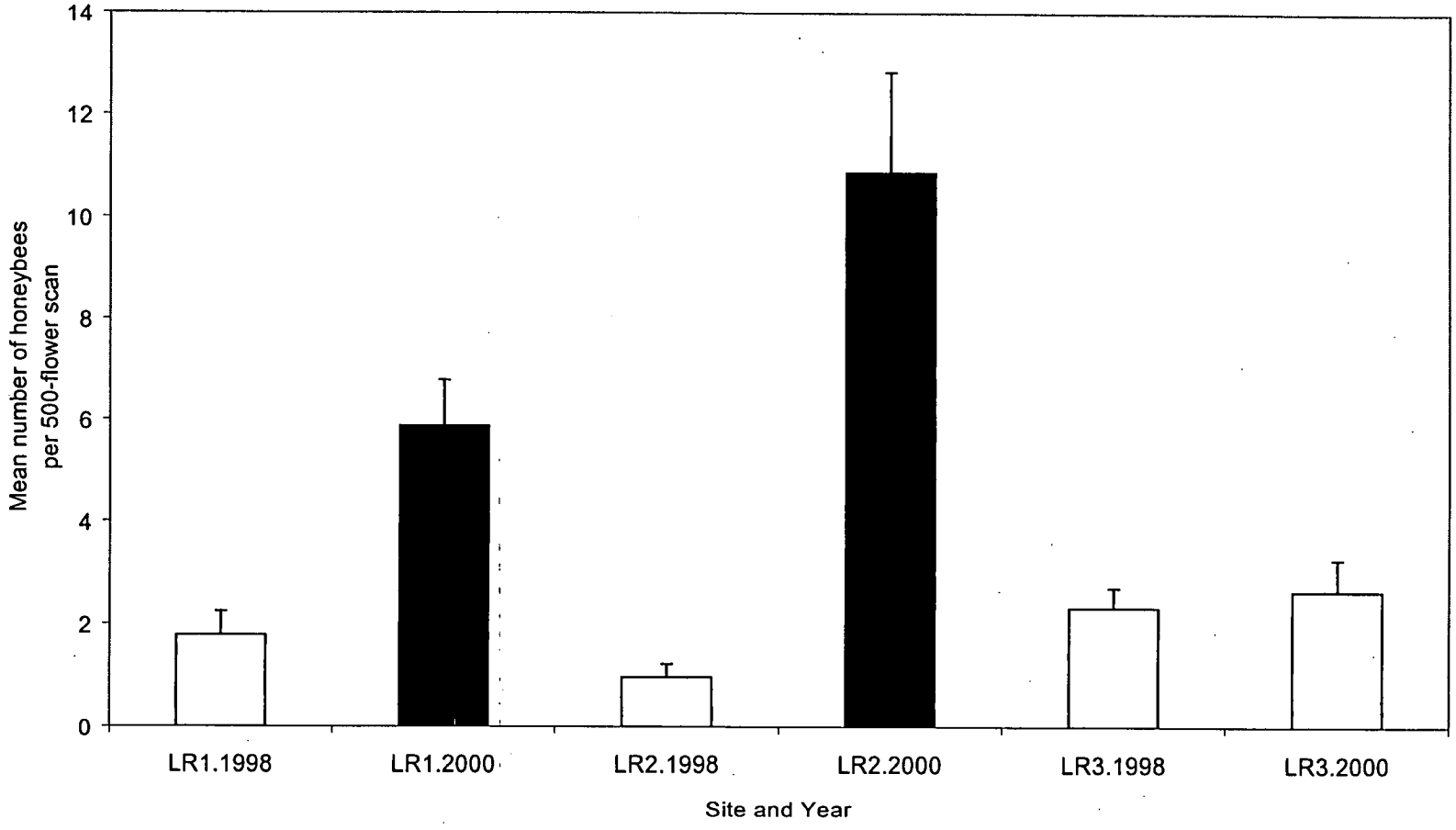

Fig. 8.3 Mean number of honeybees recorded during scans of 500 *E. lucida* flowers at the Link Road sites in 1998 (control year) and in 2000 (after introduction of apiaries). Data for different days and times of day combined. Filled bars are where apiary present, open bars are where apiary absent. n=7-13 scans for all sites. Error bars are standard errors.

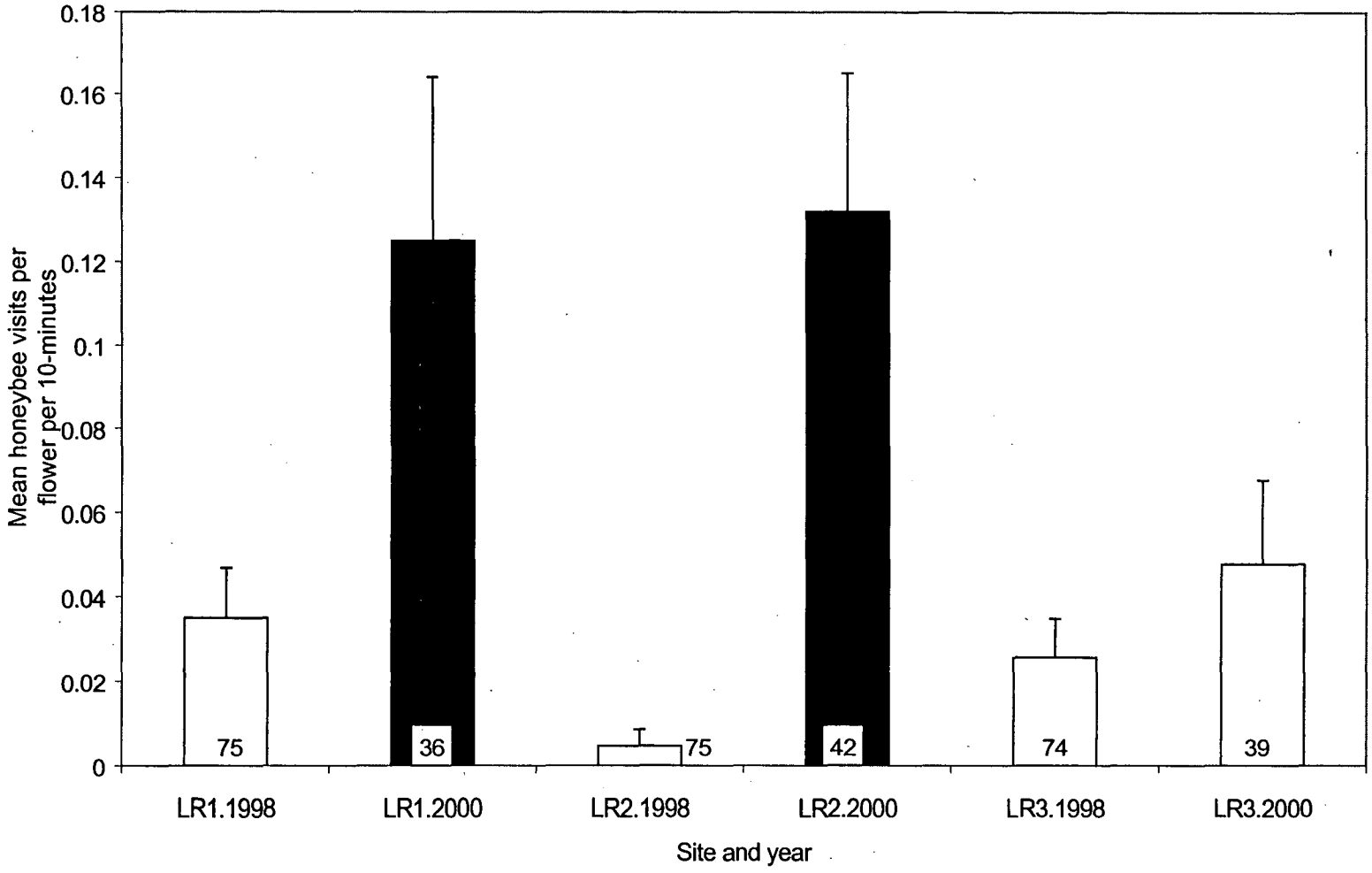

Fig. 8.4. Mean number of honeybee visits per *E. lucida* flower per 10-minutes from video footage at the Link Road sites in 1998 (control year) and in 2000 (after introduction of apiaries). Data for different days and times of day combined. Filled bars are where apiary present, open bars are where apiary absent. Sample sizes given at bottom of bars. Error bars are standard errors.

Table 8.1. Weight of floral sugar per flower (mg) for un-bagged flowers at 1000, 1300 and 1600 hrs and for bagged flowers at 1800 hrs at apiary and control sites around Tasmania. Daytime production is the difference between the 1000 hrs and 1800 hrs (bagged) nectar. Daytime consumption is the difference between the 1800 hrs (bagged) and 1600 hrs nectar. The percentage of total daytime production consumed is also shown.

Loc./Site	No. Hives	1000 hrs	1300 hrs	1600 hrs	1800 hrs (bagged)	Prod.	Cons.	% Cons.
Waratah								
WAR1	50	0.52±0.06 (59)	0.52±0.12 (59)	0.35±0.03 (60)	1.45±0.08 (40)	0.93	1.10	118.3
WAR2	60	0.31±0.05 (60)	0.29±0.04 (60)	0.37±0.05 (57)	-	-	-	-
WAR3	80	0.54±0.11 (24)	0.57±0.13 (23)	0.30±0.09 (30)	1.56±0.30 (18)	1.02	1.26	123.5
WAR4	-	0.49±0.05 (59)	0.50±0.06 (66)	0.70±0.12 (60)	-	-	-	-
WAR5	-	0.43±0.06 (59)	0.60±0.08 (58)	0.69±0.09 (61)	0.85±0.10 (31)	0.42	0.16	38.1
WAR6	-	2.15±0.32 (24)	1.61±0.22 (24)	2.51±0.50 (23)	2.58±0.46 (16)	0.43	0.07	16.3
Queenstown								
QT1	100	3.50±0.55 (65)	3.09±0.54 (45)	1.40±0.19 (46)	5.56±0.75 (32)	2.06	4.16	201.9
QT2	120	0.65±0.10 (48)	0.82±0.14 (39)	0.82±0.13 (35)	1.56±0.21 (33)	0.91	0.74	81.3
QT3	-	3.96±0.41 (64)	5.84±1.20 (46)	6.51±0.72 (39)	6.91±1.00 (31)	2.95	0.40	13.7
QT4	-	1.99±0.23 (38)	3.14±0.49 (38)	2.37±0.25 (39)	2.43±0.43 (30)	0.44	0.06	13.6
Link Road								
LR1.1998	-	1.04±0.13 (45)	1.24±0.12 (112)	1.02±0.11 (54)	-	-	-	-
LR1.2000	-	0.43±0.08 (28)	0.32±0.07 (29)	0.26±0.03 (29)	1.36±0.19 (30)	0.93	1.10	118.3
LR2.1998	-	0.79±0.07 (43)	0.97±0.08 (105)	0.85±0.11 (53)	-	-	-	-
LR2.2000	100	0.30±0.05 (30)	0.22±0.03 (30)	0.22±0.03 (30)	1.21±0.12 (30)	0.91	0.99	108.8
LR3.1998	-	0.88±0.06 (39)	1.44±0.10 (103)	1.32±0.16 (49)	-	-	-	-
LR3.2000	100	0.64±0.19 (30)	1.01±0.21 (31)	0.99±0.17 (35)	1.37±0.18 (32)	0.73	0.38	52.1

consumption of nectar also varied substantially between sites, with production ranging from < 0.5 to nearly 3 mg sugar per day, while daytime consumption ranged from < 0.1 mg to > 4 mg sugar per day (Table 8.1). The mean daytime production at apiary (1.13±0.19 mg, n=6) and control sites (0.99±49 mg, n=5) did not differ significantly (t_9=0.27, P>0.5). In contrast, daytime consumption frequently exceeded daytime production at the apiary sites, while daytime consumption was generally < 50% of production at the control sites (Table 8.1). There was a significant difference between the mean daytime consumption at apiary (1.56±0.53 mg, n=6) and control sites (0.21±0.07 mg, n=5) (t_9=2.30, P<0.05), and between the mean proportion of daytime production consumed at apiary (125.35±16.51%, n=6) and control sites (26.76±7.82%, n=5) (t_9=5.04, P<0.001).

For the Link Road study sites, the level of nectar sugar in flowers tended to increase initially then decline slightly toward the end of the day, although flowers still contained considerable amounts of sugar by late afternoon (> 0.85 mg/flower) at all three sites (Table 8.1). However, in the year 2000, nectar sugar declined steeply at the two sites which received 100 hives (LR1 and LR2). In contrast, nectar sugar at site LR3 which remained free of hives remained similar to levels in the control year (Table 8.1). There was no effect of time of day ($F_{2,857}$=1.71, P>0.1) but a significant effect of site ($F_{2,857}$=22.13, P<0.001), a significant effect of year ($F_{1,857}$=132.76, P<0.001) and a significant interaction between site and year ($F_{2,857}$=8.00, P<0.001) on the weight of nectar sugar per flower (Fig. 8.5).

Native insects at flowers

I compared the visitation rates of large dipterans and of all native insects combined between apiary and control sites (Fig. 8.6a,b). There was no effect of apiary or time of day on visitation rates by large dipterans ($F_{1,33}$=0.56, P>0.1 and $F_{2,33}$=1.34, P>0.1, respectively) or total natives ($F_{1,33}$=0.54, P>0.5 and $F_{2,33}$=1.67, P>0.1, respectively) (Fig. 8.6a,b). Visitation rates by total natives varied by more than an order of magnitude between sites, ranging from 0.02±0.01 (n=81) native insects/flower/10-minutes at QT3 to 0.37±0.08 (n=64) native insects/flower/10-minutes at WAR5 (Fig. 8.6b).

Similarly there was little apparent effect of introducing hives on native insect visitation rates at the link road sites (Fig. 8.7a,b). For large dipterans, visitation rates were not significantly different between years at the control site LR3 (t_{111}=1.43, P>0.1) or at LR1 after the introduction of 100 hives (t_{109}=0.73, P>0.4), while at LR2 visitation rates by large dipterans increased significantly after the introduction of 100 hives (t_{115}=2.13, P<0.05) (Fig. 8.7a). There was no

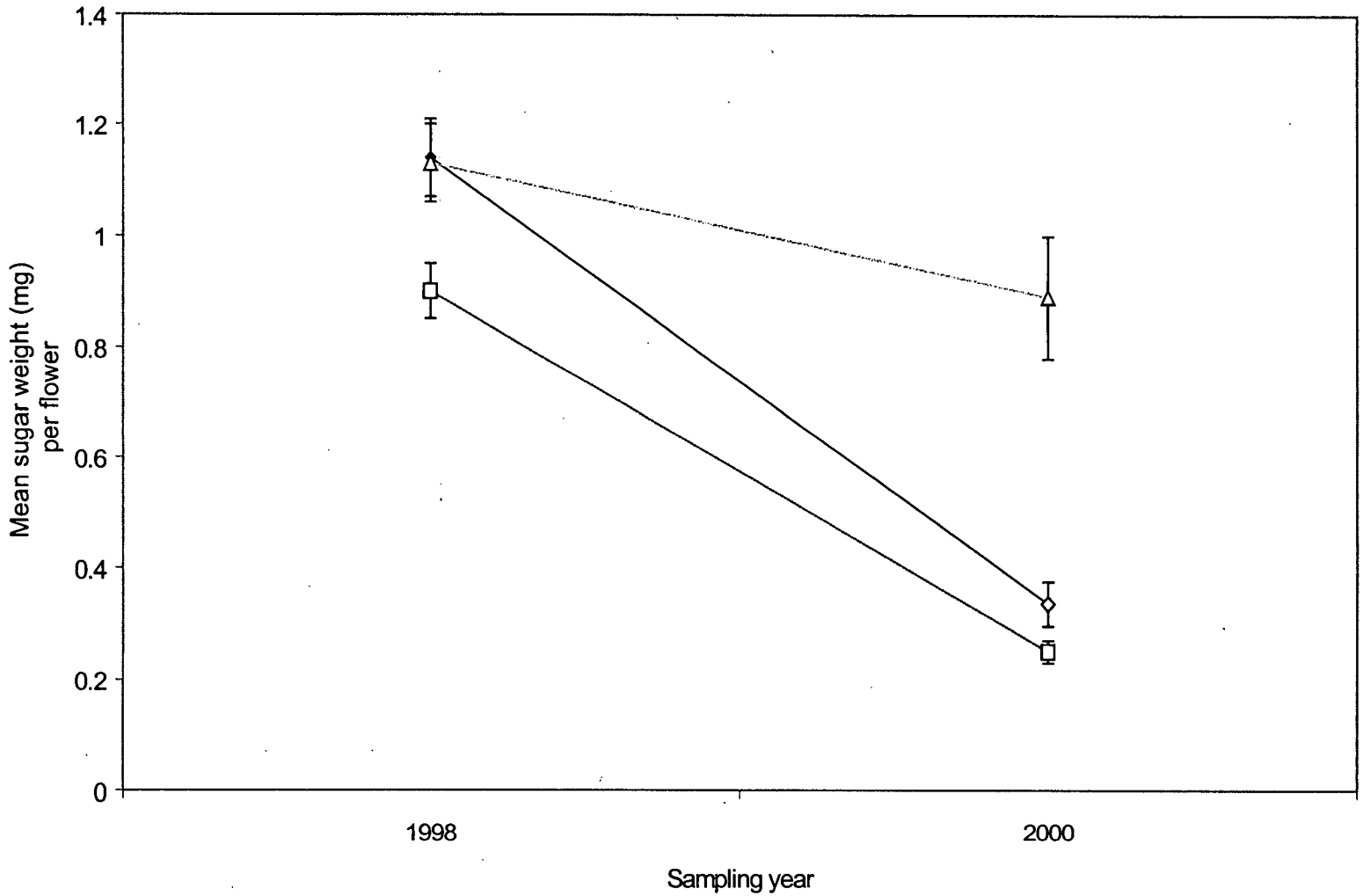

Fig. 8.5. Mean weight of nectar sugar per flower at the three Link Road sites in 1998 (no hives) and in 2000 (100 hives at LR1 and LR2). Data for different days and times of day combined. LR1 – diamonds. LR2 – squares. LR3 – triangles. Samples sizes n=191-211 in 1998. Samples sizes n=86-96 in 2000. Error bars are standard errors.

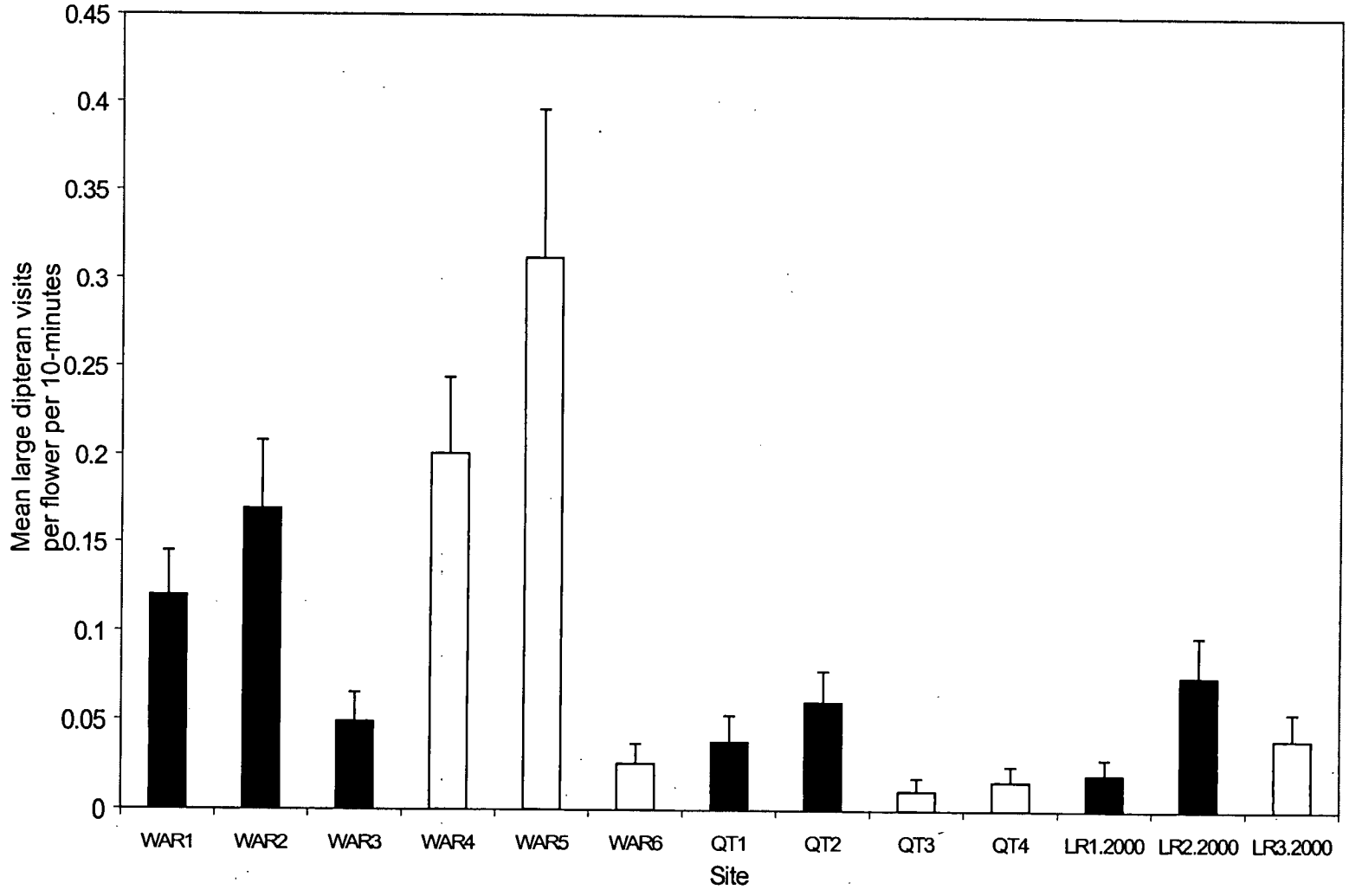

Fig. 8.6a. Mean number of large dipteran visits per *E. lucida* flower per 10-minutes from video footage at apiary and control sites. Data for different days and times of day combined. Filled bars are apiary sites, open bars are control sites. Sample sizes as for Fig. 8.2. Error bars are standard errors.

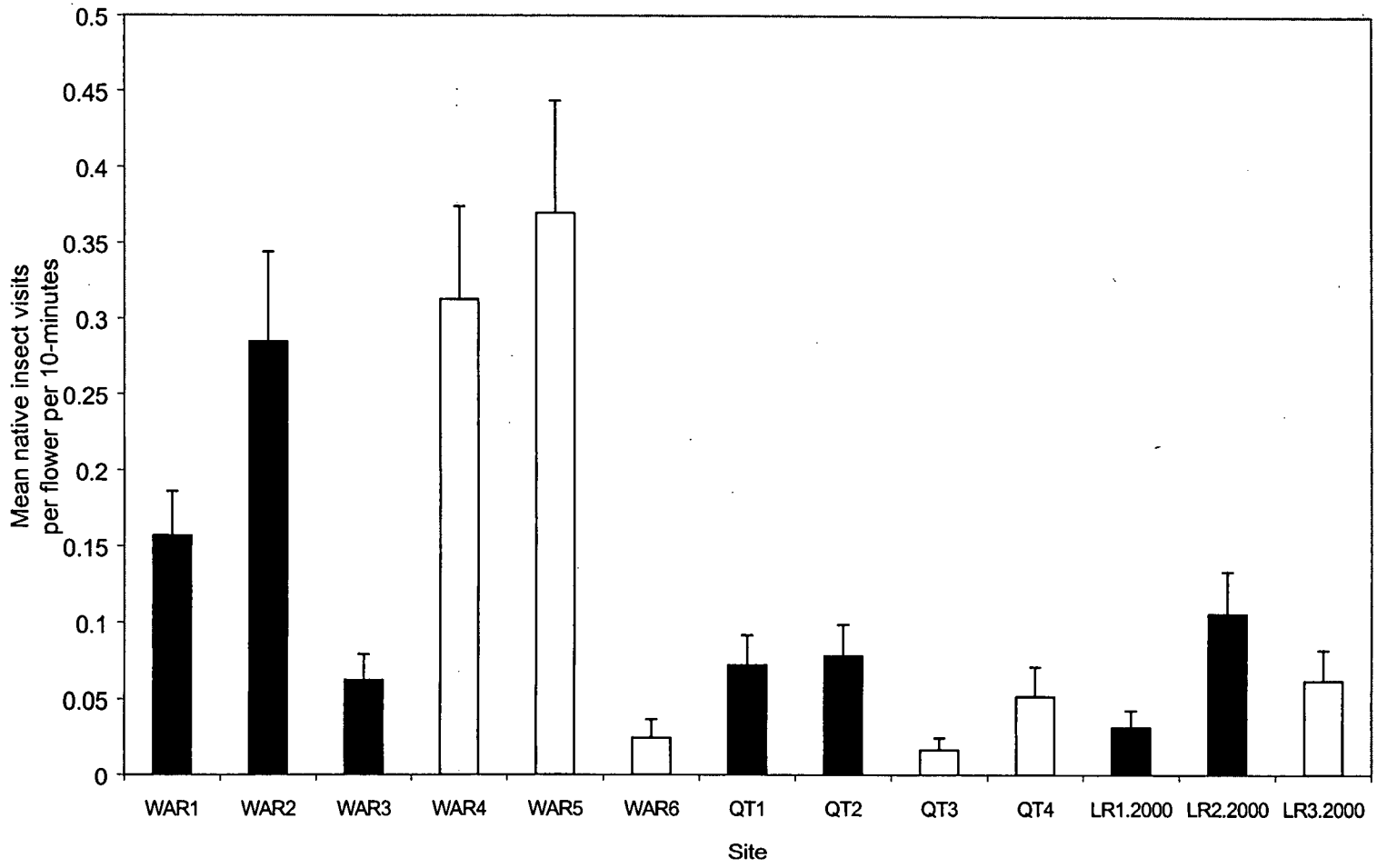

Fig. 8.6b. Mean number of native insect visits per *E. lucida* flower per 10-minutes from video footage at apiary and control sites. Data for different days and times of day combined. Filled bars are apiary sites, open bars are control sites. Sample sizes as for Fig. 8.2. Error bars are standard errors.

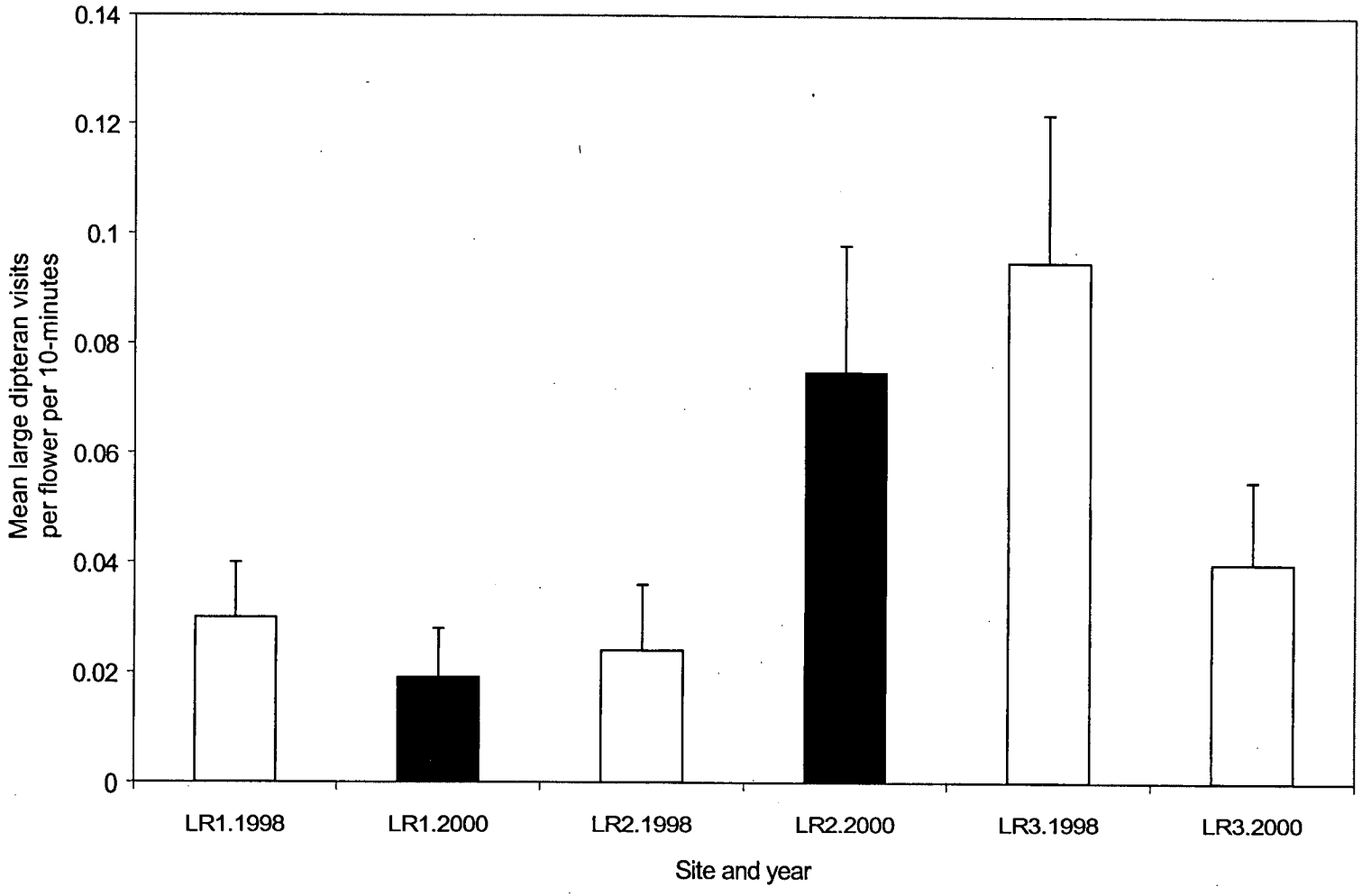

Fig. 8.7a. Mean number of large dipteran visits per *E. lucida* flower per 10-minutes from video footage at the Link Road sites in 1998 (control year) and in 2000 (after introduction of apiaries). Data for different days and times of day combined. Filled bars are where apiary present, open bars are where apiary absent. Sample sizes as for Fig. 8.4. Error bars are standard errors.

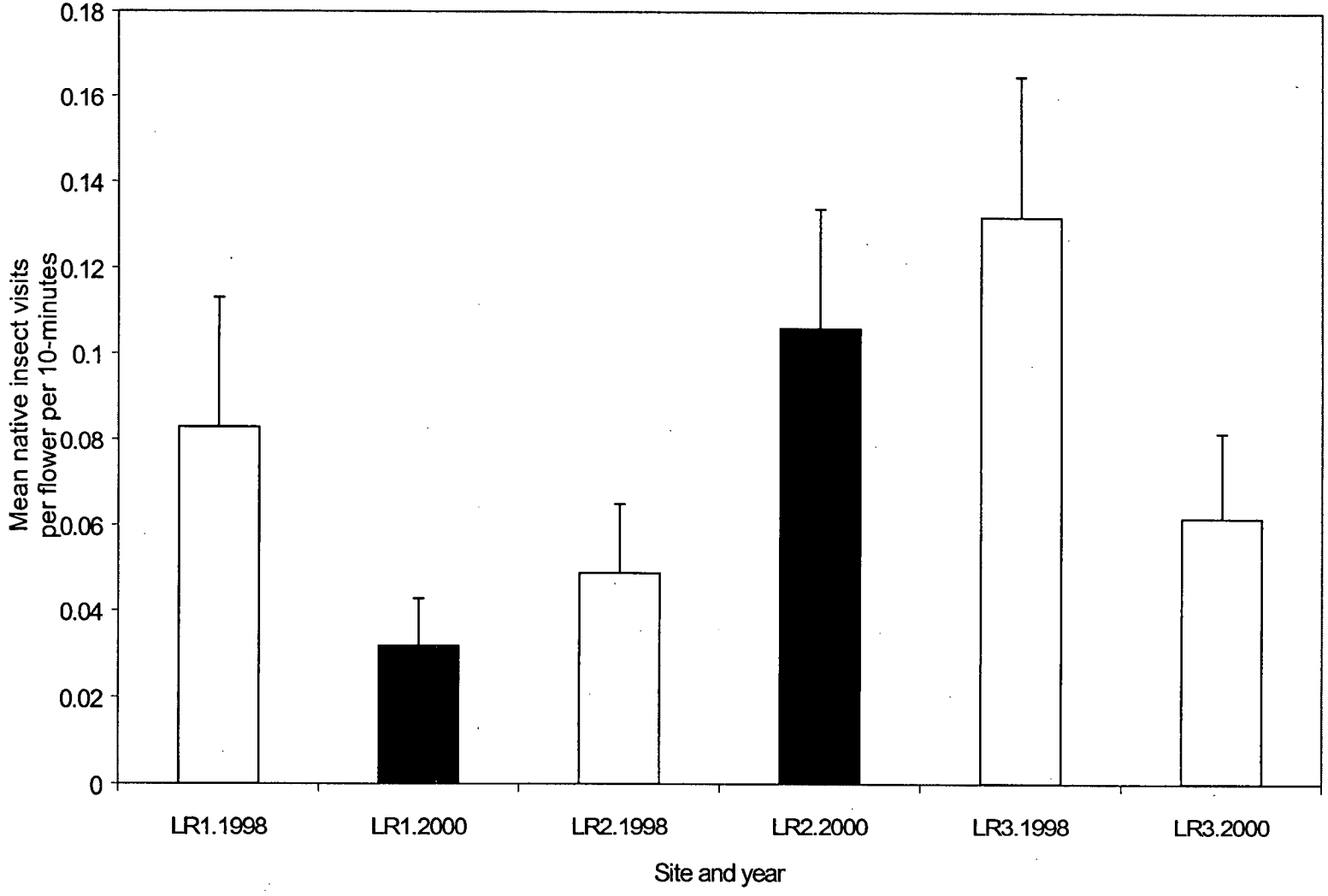

Fig. 8.7b. Mean number of native insect visits per *E. lucida* flower per 10-minutes from video footage at the Link Road sites in 1998 (control year) and in 2000 (after introduction of apiaries). Data for different days and times of day combined. Filled bars are where apiary present, open bars are where apiary absent. Sample sizes as for Fig. 8.4. Error bars are standard errors.

significant difference in the visitation rate by total natives at LR1 and LR2 in the second year after the introduction of hives (t_{109}=1.16, P>0.2 and t_{115}=1.93, P>0.05, respectively), or at the control site LR3 in the second year (t_{111}=1.46, P>0.1) (Fig. 8.7b).

Feral honeybees

I noted the colour of honeybees visiting *E. lucida* flowers on the video footage and classified bees as either hive (those with a golden coloured abdomen) or feral bees (those with a darker or black abdomen). Of a total of 76 dark-coloured (presumed feral) honeybees recorded during total video footage, only 6 were recorded at apiary sites. Assuming approximately equal sampling effort at apiary and control sites (the number of 'flower-hours', i.e. total video segments multiplied by total number of flowers observed, was 666.7 and 622.8 at apiary and control sites, respectively), there were significantly fewer feral honeybees recorded at apiary sites than expected by chance (X^2_1=53.9, P<0.01). For the Link Road sites, 100% of honeybees recorded at all three sites were dark coloured in the first year when all sites were free of hives. At the control site LR3, 100% of honeybees were also dark coloured in the second sampling year. In contrast, 100% and 86% of honeybees recorded at LR1 and LR2, respectively, were golden coloured in the second year after the introduction of 100 hives.

Native insects on sticky traps

The mean number of large dipterans caught on sticky traps varied widely between sites, ranging from 0.15±0.10 (QT1) to 2.73±0.69 large dipterans per trap (WAR3), while the total number of insects per sticky trap was relatively consistent between sites (Fig. 8.8a,b). For all sites, there was no effect of apiary on the number of large dipterans (t_{11}=0.55, P>0.5) or total native insects (t_{11}=1.70, P>0.1) caught on sticky traps. Similarly there was no apparent effect of introducing hives on native insect visitation rates at the link road sites (Fig. 8.9a,b). The number of large dipterans caught on sticky traps was not significantly different between years at the control site LR3 (t_{30}=1.88, P>0.05) or at LR1 (t_{29}=0.86, P>0.1) and LR2 (t_{36}=0.41, P>0.5) after the introduction of 100 hives (Fig. 8.9a). Similarly, the total number of insects caught on sticky-traps was not significantly different between years at the control site LR3 (t_{30}=0.27, P>0.5) or at LR1 (t_{29}=0.58, P>0.5) and LR2 (t_{36}=0.54, P>0.5) after the introduction of 100 hives (Fig. 8.9b).

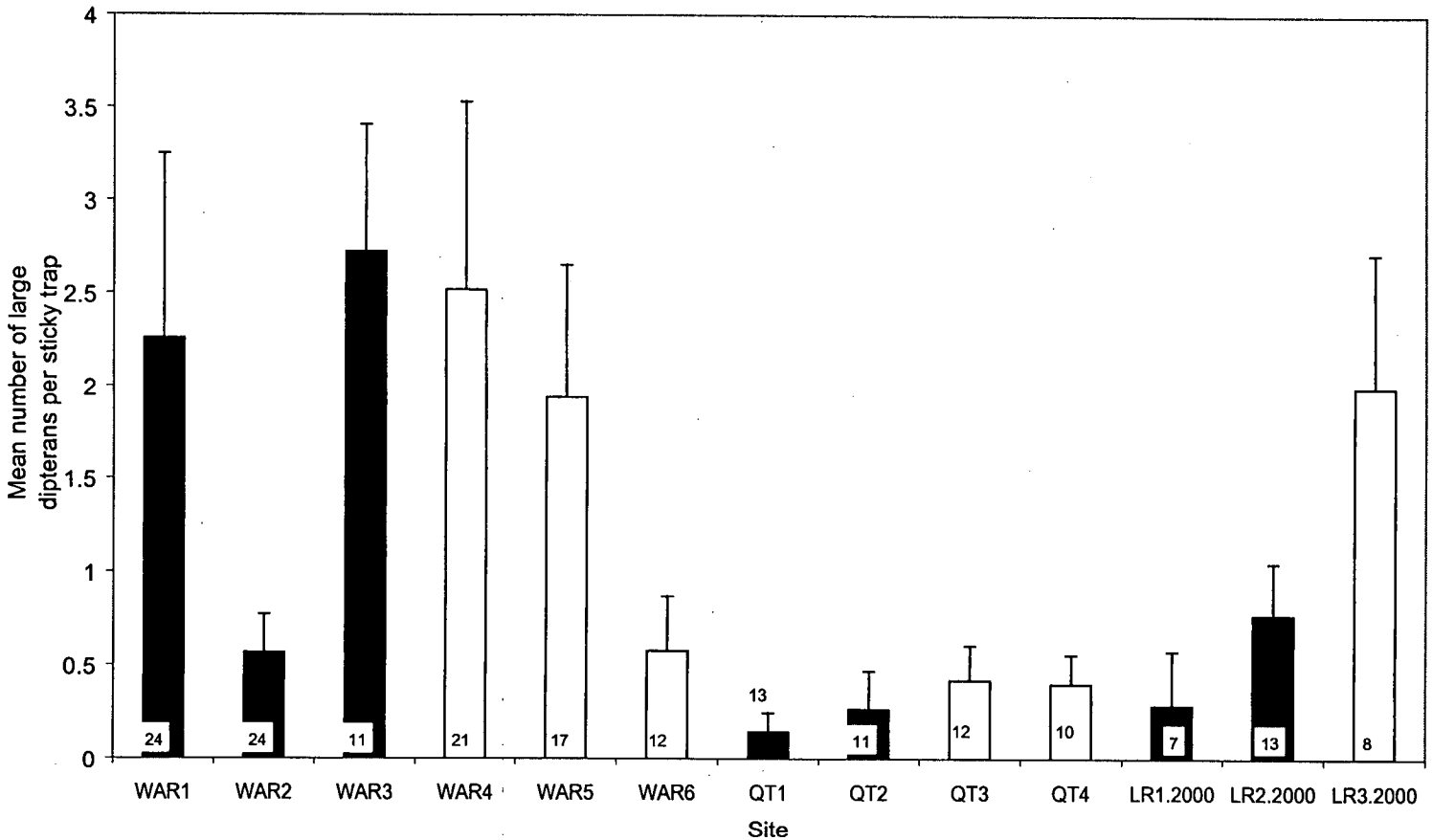

Fig. 8.8a. Mean number of large dipteran per sticky trap at apiary and control sites. Filled bars are apiary sites, open bars are control sites. Sample sizes given at bottom of bars. Error bars are standard errors.

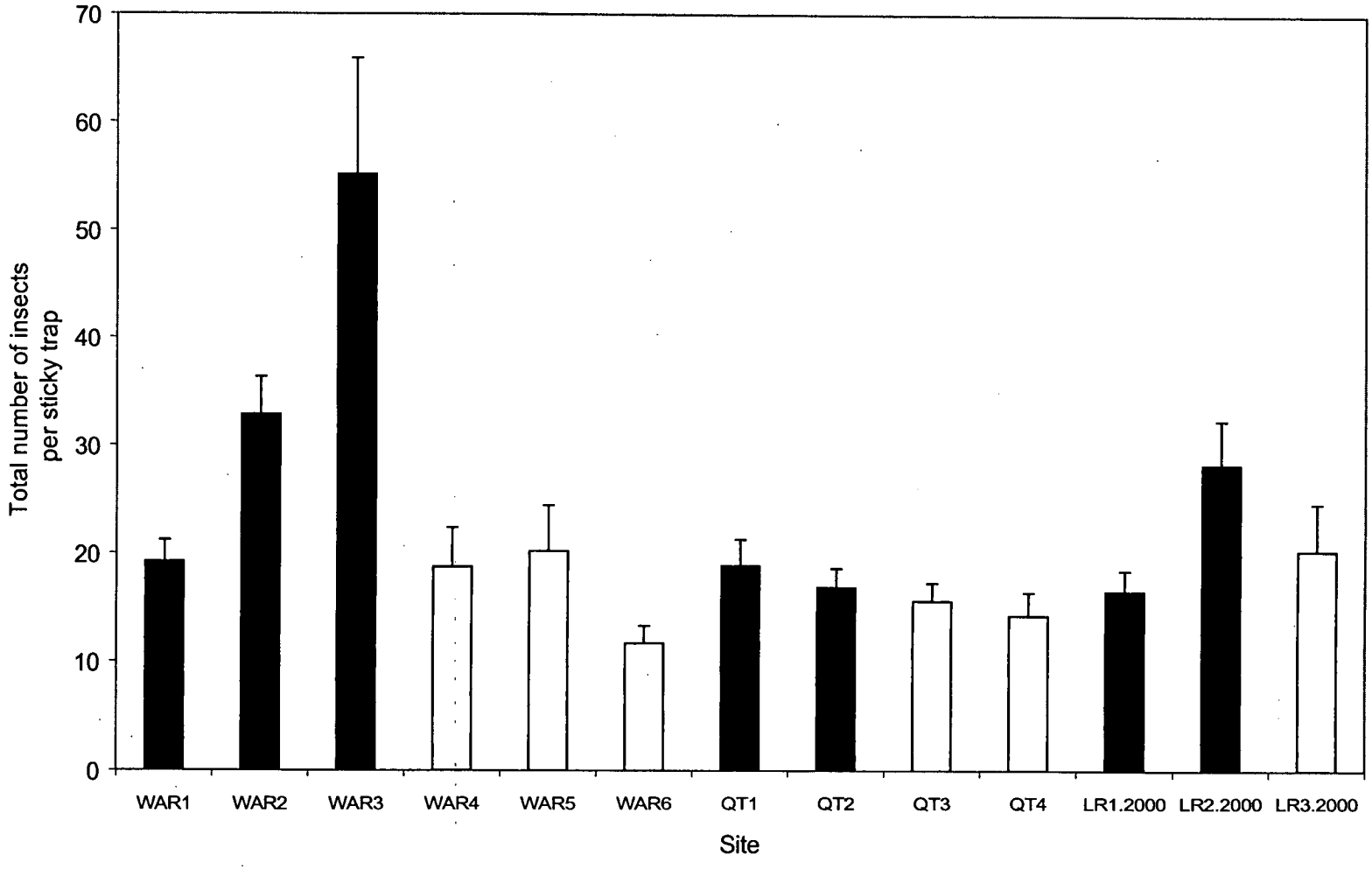

Fig. 8.8b. Mean number of native insects per sticky trap at apiary and control sites. Filled bars are apiary sites, open bars are control sites. Sample sizes as for Fig. 8.8a. Error bars are standard errors.

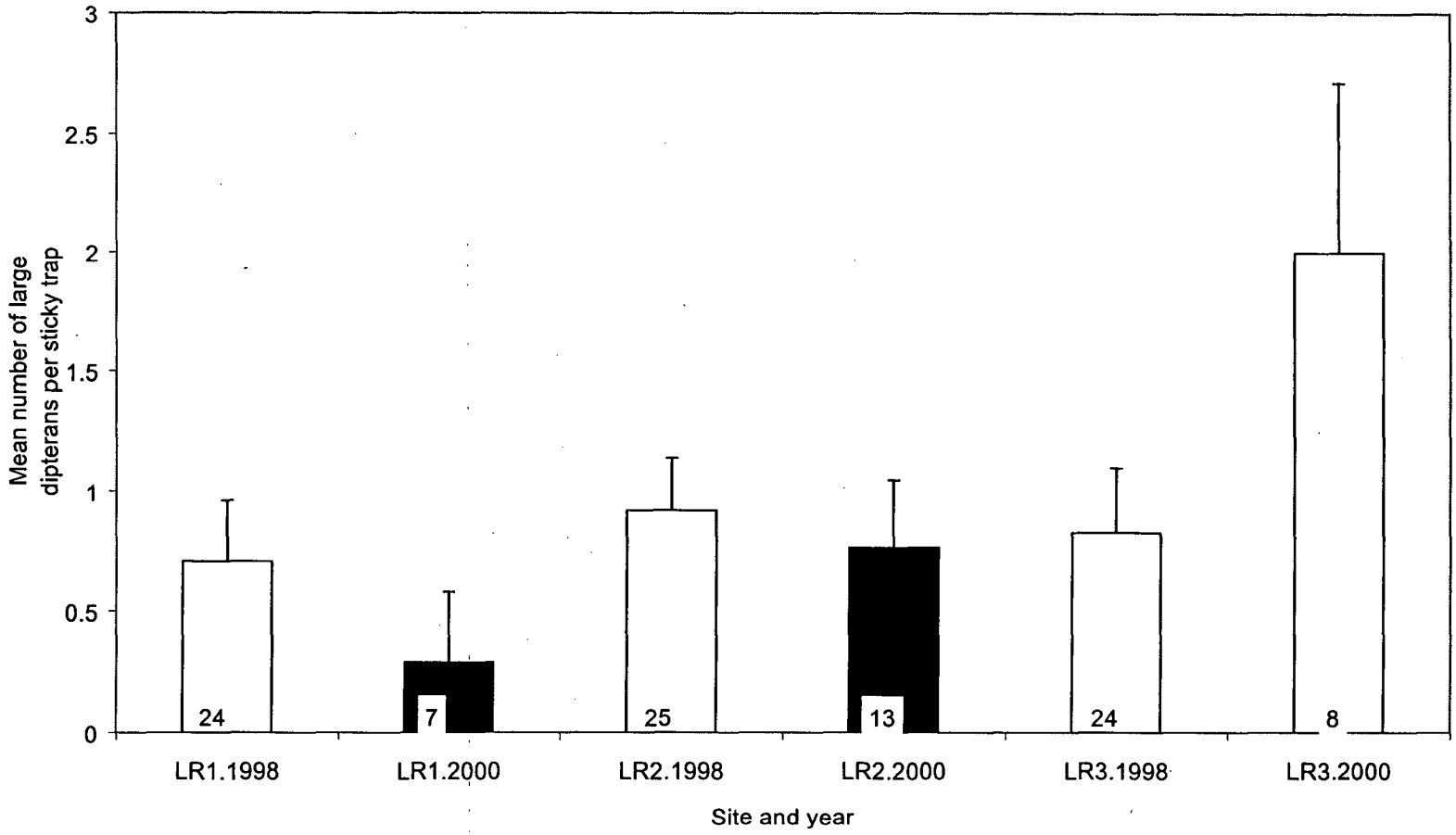

Fig. 8.9a. Mean number of large dipteran per sticky trap at the Link Road sites in 1998 (control year) and in 2000 (after introduction of apiaries). Filled bars are where apiary present, open bars are where apiary absent. Sample sizes given at bottom of bars. Error bars are standard errors.

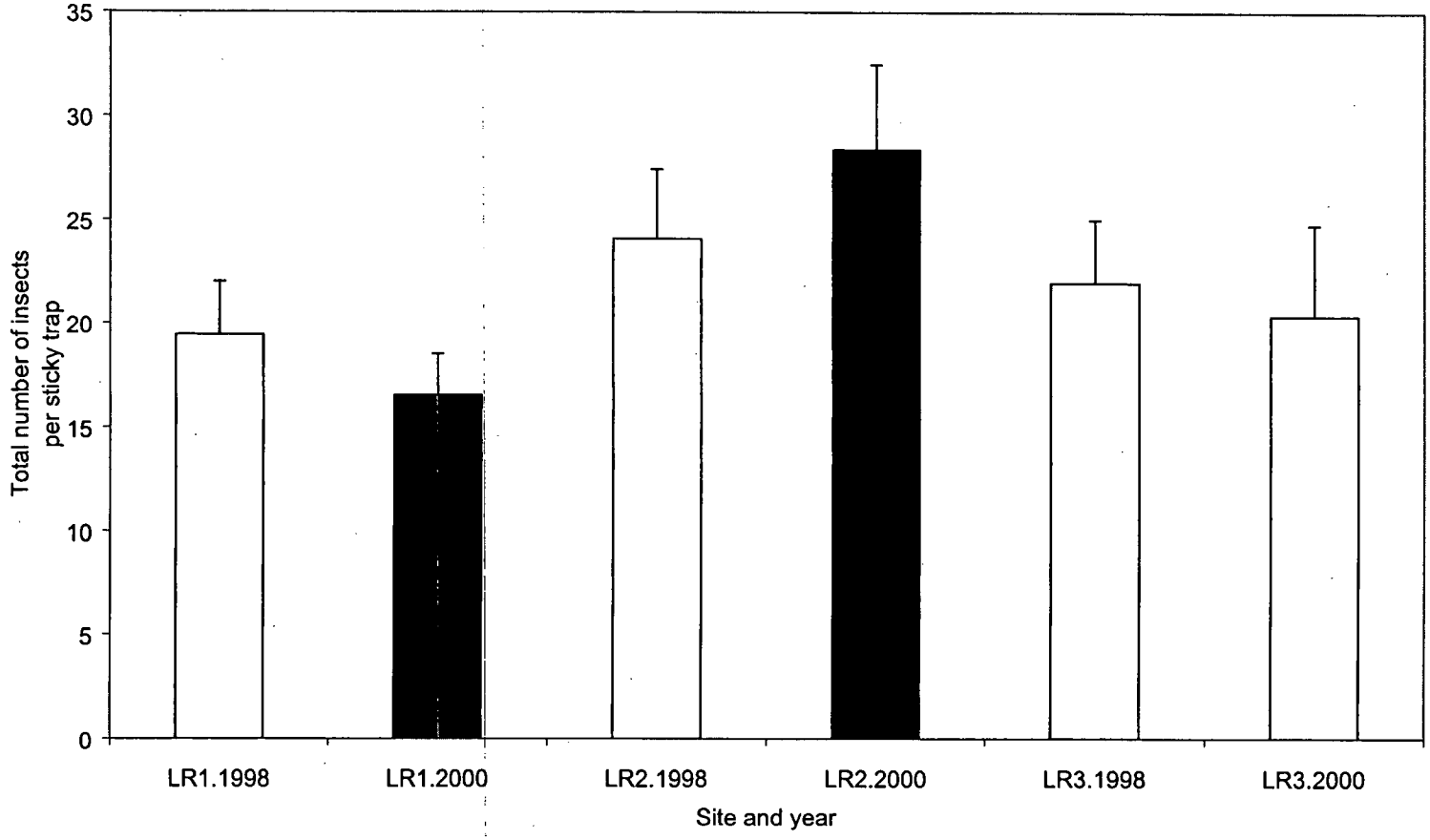

Fig. 8.9b. Mean number of native insects per sticky trap at the Link Road sites in 1998 (control year) and in 2000 (after introduction of apiaries). Filled bars are where apiary present, open bars are where apiary absent. Sample sizes as for Fig. 8.9a. Error bars are standard errors.

For all sites, there was a weak correlation between the mean number of large dipterans caught on sticky traps and the mean visitation rate by large dipterans to *E. lucida* flowers ($F_{1,14}$=3.44, r^2=0.20, 0.05<P<0.1; Fig. 8.10).

Discussion

The agistment of commercial loads of honeybee hives into cool temperate rainforest clearly resulted in an increase in the activity of honeybees at *E. lucida* flowers, with the number of honeybees recorded at flowers increasing by as much as 26 fold following the introduction of an apiary to one of the Link Road sites. For all sites combined, honeybee activity was 2.5 times greater at apiary sites compared to control sites. Given the very large number of honeybees involved in even moderately sized apiaries (with up to 60 000 per hive), this increase seems surprisingly modest, and presumably reflects significant numbers of feral honeybees at control sites and the distribution of hive bees over a large foraging area (*cf.* Paton 1993). A number of other studies have monitored honeybee activity after the introduction of commercial hives. Paton (1993) noted an increase in honeybee activity at *Callistemon rugulosus* flowers with proximity to a large commercial apiary and after the introduction of 10 hives to an experimental plot. Similarly, Gross and Mackay (1998) noted an increase in honeybee visitation rates to *Melastoma affine* after the introduction of additional hives near their study area, while Roubik (1978) increased the activity of africanized honeybees at target flowers by introducing 2-5 swarms to experimental sites. Ettershank and Ettershank (1992) also recorded a modest increase in honeybees at *E. lucida* flowers near commercial apiaries compared to control areas, although the latter were < 1 km from apiary sites.

Although the increase in honeybee activity in the vicinity of an apiary tended on average to be relatively modest, the increased numbers of hive bees significantly reduced the availability of nectar sugar in *E. lucida* flowers. This impact was most pronounced in terms of nectar consumption at sites. On average, insects consumed 125% of the total diurnal production of nectar at apiary sites, while insects consumed only *ca* 25% of the total diurnal production of nectar at control sites. The patterns of nectar availability varied substantially among sites; however, nectar levels tended to remain relatively low throughout the day at apiary sites, presumably due to the increased consumption by hive bees. In contrast, flowers at control sites tended to contain greater accumulations of nectar, and nectar levels tended to increase between the morning and afternoon sampling sessions (Table 8.1). *E. lucida* flowers at the most productive control site, QT3, contained a mean of > 6 mg of sugar by the late afternoon; this accumulated nectar frequently puddled at the base of flowers

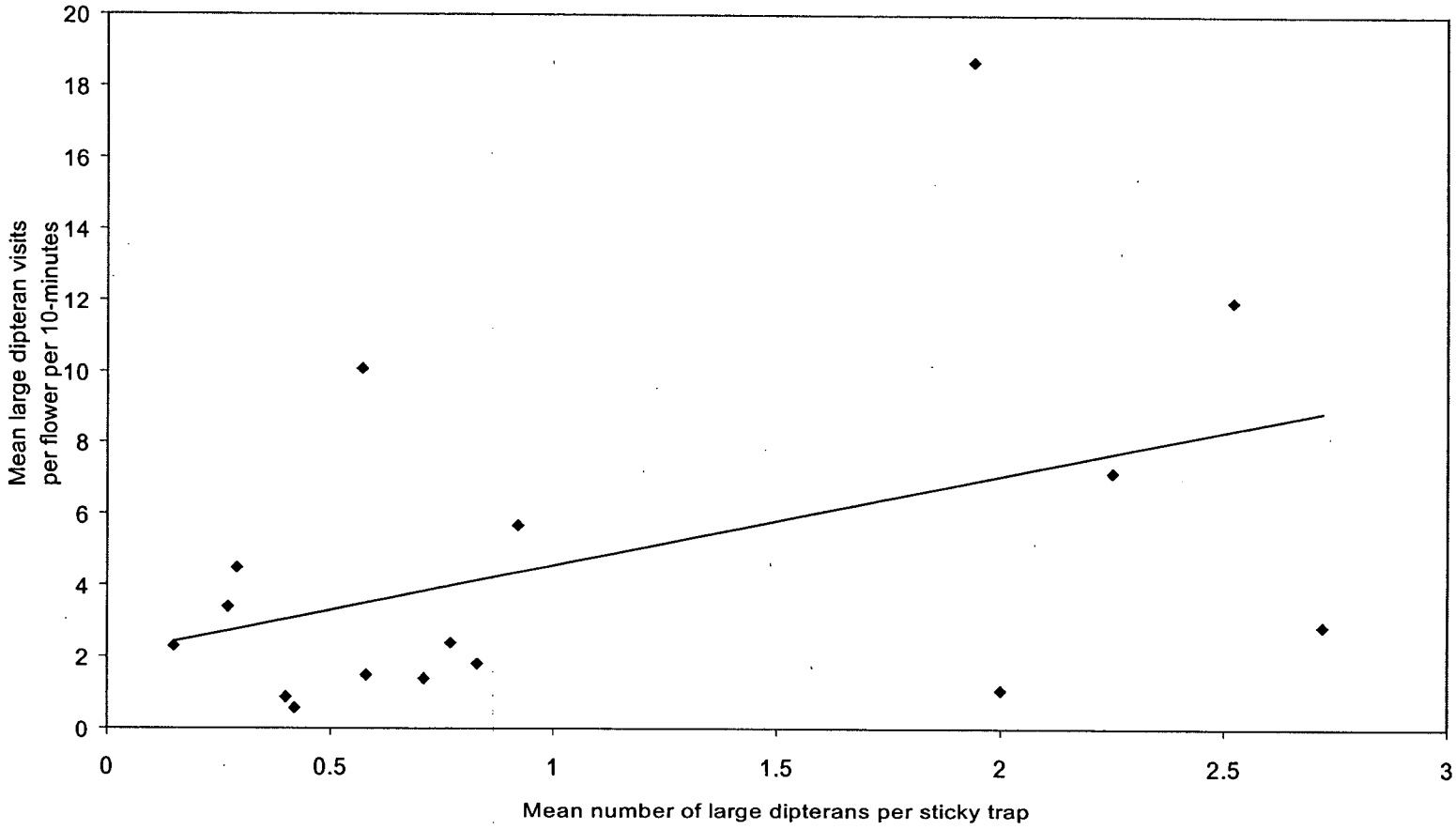

Fig. 8.10. Regression of the mean number of large dipterans per flower per 10-minutes against the mean number of large dipterans per sticky trap for the thirteen sites. Linear regression line is shown.

and was occasionally observed dripping onto the ground. The effect of hive bees on nectar levels was also readily apparent at the Link Road sites. In the control year, nectar levels were similar at all three sites (Table 8.1). At the two apiary sites LR1 and LR2, nectar levels were significantly reduced in the second year after the introduction of 100 hives to each site. In contrast, nectar levels at the control site were similar in both years, indicating the reductions in nectar at the apiary sites were not the result of differences between sampling years.

Honeybees are highly efficient foragers, and bees from commercially managed hives might be expected to consume significant quantities of the available nectar resource. In a recent review of research into honeybee impacts, Paton (1996) concluded that honeybees typically consume > 80% of available nectar and that the potential for resource competition with native anthophilous species is high. Unfortunately, few studies into honeybee impacts have monitored background resource (i.e. nectar and pollen) levels, making it difficult to interpret any observed shifts in the foraging activity or abundance of native anthophiles resulting from the introduction of hives (e.g. Sudgen and Pyke 1991; Schwarz and Hurst 1997).

In the present study, honeybees made up 51.3±5.4% (range = 31.4 – 75.7%) of total visits to flowers at apiary sites (see Chapter 3, Table 3.2), and their contribution to nectar consumption is presumed to be at least this high. However, despite the increased foraging pressure from hive honeybees at apiary sites and the resulting reduction in available nectar sugar in flowers, I found no evidence that commercial loads of honeybees reduced the visitation rate or abundance of native anthophilous insects. One reason for this might be that visitation rates by the native pollinators varied enormously between control sites (Fig. 8.6a,b), making it difficult to statistically demonstrate any potential effect of the presence of an apiary (see Appendix 2). However, the data from the before and after experiment at the Link Road provided unequivocal evidence that introducing hives to a site does not necessarily result in a decline in native insect activity or abundance (Fig. 8.7a,b and 8.9a,b).

Data from the present study suggest that the native insect fauna at control sites were not utilising all of the available nectar resource. That is, *E. lucida* flowers appeared to produce a surplus of nectar. Even at the two control sites where visitation rates by native insects were relatively very high (WAR4 and WAR5; Fig. 8.6a,b) and nectar levels were maintained at relatively low levels, the trend was still for nectar to increase rather than to decline over the day (Table 8.1). Furthermore, at control sites where visitation rates by native insects were extremely low (e.g. QT3), there was clearly a large surfeit of nectar produced which was not being utilised by the native fauna. The presence of un-

exploited nectar at sites without apiaries provides one explanation why introducing hives may not necessarily result in a decline in native insects at flowers, as hive honeybees may be removing only this surplus nectar.

The majority of studies in which nectar levels have been monitored under conditions where birds and/or insects have been active have recorded a decline in available nectar in open flowers, often to very low levels (e.g. Bond and Brown 1979; Ford 1979; Collins *et al.* 1984; Paton 1985, 1993, 1996). Such a result would appear to be consistent with ecological theory that niche space (such a food resource) should be fully utilised by coexisting species (Hutchinson 1959; Heinrich 1976; Pyke 1990). In a study into the impacts of honeybees on honeyeaters foraging on *C. rugulosus*, Paton (1993, 1996) monitored background nectar levels and found that all the nectar produced by the plants was being consumed by honeyeaters and/or honeybees. Under these conditions of resource limitation, increasing honeybee numbers resulted in an increase in the birds' feeding territories to compensate for a reduction in nectar availability per inflorescence (Paton 1993, 1996). In contrast, in a second study into hive impacts during overwintering in *Banksia ornata* heathland, Paton (1996, 1999) found that although honeybees depressed *B. ornata* nectar levels, inflorescences still contained substantial quantities of un-utilised nectar within 100 m of a commercial apiary, while at sites without hives, *B. ornata* inflorescences contained in excess of 1.5 g of sugar per inflorescence. As expected under such conditions of resource excess the introduction of hives had no discernable effect on the numbers of honeyeaters, small nectarivorous mammals or invertebrates (Paton 1996, 1999).

The results of the present study support the finding of Paton (1996, 1999) that surpluses of nectar do occur, and that they may limit or negate any potential impacts of introducing commercial hives to a site. However Paton attributed the existence of a surfeit of un-exploited resource in *B. ornata* heathlands to an artificial deficiency in the native pollinator fauna, principally native honeyeaters, caused by the destruction of the birds' summer and autumn woodland habitat. The absence of any impact of hives on *B. ornata* therefore reflects previous disturbance to natural systems, rather than to hive honeybees being a neutral introduction *per se* (Paton 1996). The reasons for the enormous variation in native insect visits to *E. lucida* flowers observed in the present study, and for the apparent surplus of nectar produced by *E. lucida* flowers, are not known. However, they are unlikely to be due to any artificial disruption to the abundance of native pollinators as the experimental sites in the present study were located within very large stands of effectively pristine forest.

Whatever the underlying reasons for the apparent nectar surplus, the existence of a superabundance of nectar in *E. lucida* forest is clearly capable of dampening any adverse effects of commercial hives on the native insect fauna. However, it is possible that introducing commercial hives exclusively to areas with high background levels of native insects and concomitant low levels of nectar (e.g. WAR1 and WAR2 in the present study) might cause a statistically demonstrable reduction in native insect visitors (see Appendix 1). Furthermore, the results of the present study are from flowers <2.5 m above ground level. It is possible that native insects occur in greater numbers on flowers higher in the canopy. If so, the nectar surpluses observed in flowers near the base of trees may not be as prevalent in higher flowers, and resource competition may be more likely in the canopy proper. Further work is required to determine whether hive bees may impact on native insect visitors to *E. lucida* under conditions other than those of the present study.

A curious finding of the present study was that dark-coloured (presumed feral) honeybees appeared to be largely excluded from apiary sites. While the colour difference between hive and feral bees is not always clear and some honeybees may have been mis-classified, the difference in the number of dark-coloured honeybees recorded at apiary and control sites was very pronounced. For example, dark-coloured (presumed feral) honeybees made up > 80% of honeybees recorded at control sites, while at apiary sites dark-coloured honeybees made up only 2% of total honeybees recorded. Similarly, at the Link Road, the introduction of 100 hives to two sites resulted in a 100% decline in the proportion of dark-coloured bees at one site (LR1) and in an 87% decline at the other (LR2). These results strongly imply that hive bees were competitively excluding feral bees from the vicinity of apiaries.

If, as suggested above, the absence of any impact of hive bees on native insects is attributable to a superabundance of nectar, it is difficult to envisage how hive bees can have been competitively excluding feral honeybees through resource competition. However, it is possible that hive bees excluded feral honeybees via mechanisms other than resource competition. From the entire 199 hours of video footage I observed only six examples of two honeybees physically interacting at flowers (see Chapter 6), and direct competitive interference is considered unlikely as an explanation for the exclusion of feral honeybees from apiary sites. However, feral honeybees may have learned to avoid areas or trees supporting large numbers of hive bees, possibly through very occasional aggressive contact leading to subsequent avoidance of a resource patch. For example, Johnson and Hubbell (1975) found that two species of eusocial *Trigona* bees partitioned plants of *Cassia biflora*, with the

more aggressive bee excluding the other species from clumped patches by a low but continual aggressive displacement at flowers. Alternatively, hive bees may have exerted a subtle form of interference on feral honeybees through the large numbers of hive honeybees recruiting to flowers. For example, Roubik (1980) observed such a phenomenon in his study of aggressive interactions between Africanized honeybees and native stingless bees at artificial feeders. While Africanized honeybees were not overtly aggressive toward native *Trigona* bees, their large size, rapid recruitment to a resource in large numbers and comparatively rapid movement at flowers appeared to have significant interference value for the invading honeybee (Roubik 1980). A similar, subtle form of interference may have occurred between hive and feral honeybees in the vicinity of the apiary sites in the present study.

Chapter 9. Impacts of hive honeybees on *E. lucida*: II. Pollen removal, pollen deposition, and fruit and seed set

Abstract

I examined the impacts of hive honeybees on the reproductive performance of *E. lucida* by comparing rates of pollen removal, pollen deposition, and fruit and seed set at sites within 400 m of a commercial apiary with control sites > 2 km from the nearest apiary. Hive bees removed pollen more rapidly from flowers in the vicinity of apiaries, and there was a significant reduction in the overall 'standing crop' of pollen in male flowers around apiaries. A mean of 4.46±0.40% of anthers in a flower carried pollen at apiary sites compared to 22.39±1.08% at control sites. However, there was no difference in the number of pollen grains deposited on stigmas at apiary and control sites (12.47±1.41 and 15.12±1.61 pollen grains per stigma section at apiary and control sites, respectively). Fruit set tended to be higher around apiaries (74.27±5.91% and 46.58±5.13% at apiary and control sites, respectively). However, there was no difference in the percentage of fruit which fully dehisced (59.26±5.23% and 55.68±5.60%), fruit weight (0.091±0.002 g and 0.081±0.002 g) or seed set (35.82±0.96% and 28.53±1.31%) between apiary and control sites, respectively. Therefore, despite removing pollen more rapidly and reducing the standing crop of pollen available in flowers, hive honeybees appear to have little net impact on the female reproductive performance of *E. lucida*.

Introduction

Introduced honeybees may impact on a native plant either indirectly, by affecting the abundance or activity of the native pollinators, or directly by altering rates of pollen removal and deposition through their own behaviour as pollen vectors (Pyke 1990). Whether honeybees have a net impact on a plant species' reproductive success will therefore depend on their effectiveness relative to the native pollinators, and whether any detrimental effect on the native pollinator service are compensated for by the introduced honeybee's activity. The effect of honeybees may be: (a) negative, for example through displacing native pollinators and not providing a compensatory service (Pyke 1990) or by reducing the availability of pollen to legitimate pollinators (e.g. Pyke 1990; Paton 1993, 1997); (b) nominally positive by increasing pollen deposition and fruit and/or seed set (e.g. Paton 1997, 1999); or (c) effectively neutral through a balancing out of these various processes.

The only study to date on honeybee impacts in Tasmania involved a cursory examination of the potential impacts of hive honeybees on native insects associated with *E. lucida,* but did not consider potential impacts on *E. lucida* fruit and seed set (Ettershank and Ettershank 1992; Ettershank 1993). This chapter reports on the impacts of hives on pollen removal, pollen deposition, and fruit and seed set in *E. lucida.* The impacts of hives on honeybee numbers, nectar levels, and the abundance and activity of native insects at flowers are considered in Chapter 8.

Methods

Experimental design and study sites

I compared the reproductive performance of *E. lucida* at seven apiary sites (War1-6, QT1-2 and LR1-2) and six control sites (WAR4-6, QT3-4 and LR3). See *Study Sites* section for details of sites.

Pollen removal

E. lucida flowers typically bear between 80-140 anthers (mean±se = 108.46±4.17 anthers/flower, n=40, range 60–184; measurement taken at MAY in 1999). Anthers are pink before they dehisce, white after dehiscence and while still bearing pollen, and brown once pollen has been removed. These colour changes provide an efficient means of estimating the quantity of pollen remaining in flowers, as the approximate percentage of pollen-bearing anthers in a flower can be rapidly assessed by eye.

I examined the effect of apiaries on the removal of pollen from flowers in two ways. First, I scored the percentage of white anthers in a sample of 10-15 male flowers taken from 3-10 trees at each site (range of n=30-150 flowers per site). I selected male flowers by avoiding those with the style fully exerted (i.e. in the female phase). However because the style does not exert in all female-stage flowers in *E. lucida* (see Chapter 2), some female flowers may have been inadvertently included in the samples. All flowers were scored after a 2-3 day period of warm clear weather. I sampled all the Waratah sites on the same day in mid February 1999, all the Queenstown sites on the same day in mid January 2000, and all the Link Road sites on the same day in late January 2000.

Second, I followed the pattern of pollen availability in flowers of known age. At each site a sample of 10 flowers on three trees (n=30 flowers per site) were tagged with a loop of coloured wire at late bud-cap stage (i.e. just prior to flower opening: Day 0). I then scored the percentage of white anthers in these tagged flowers on Days 3, 5, 7 and 10. I sampled all the Queenstown sites on the same days in mid January 2000, all the Link Road sites on the same days in

late January 2000, and all the Waratah sites with the exception of WAR1on the same days in mid February 2000. WAR1 was not included as the hive bees had ceased foraging at this site because all available hive supers had been filled and not replaced by the apiarist.

Pollen deposition

I used the flowers of known age from the previous experiment to examine pollen deposition at apiary and control sites. On Day 10 and after flowers had been scored for the percentage of white anthers, the stigma lobes (range of 5-8 lobes per stigma) of each tagged flower were excised with fine scissors and placed on a microscope slide in a drop of lacto-phenol blue. Stigma lobes were left for several minutes to absorb the stain and then lightly squashed under a coverslip and sealed with clear nail-varnish. The total number of viable pollen grains (those that had absorbed stain; Ramsey and Vaughton 2000) were counted under 400x magnification for each stigma lobe for the first (distal) field of view (one field of view= 448-μm segment of stigma lobe). The number of pollen grain on this distal section of stigma lobe was used as an index of pollen deposition for comparisons between apiary and control sites.

Fruit and seed set and fruit weight

I examined fruit set at WAR1, WAR2, WAR4 and WAR5 and at QT1 and QT3. In the summer of 1999, I tagged 30 flowers in random stages of anthesis on five trees (n=150 flowers per site) using a loop of colored wire tied to the pedicel of the leaf associated with each flower. Flowers were tagged on the leaf rather than the flower pedicel as the latter are lost with aborted flowers/fruits. The total number of tags still present on trees and the number of these tagged flowers which had matured fruits were scored 12 months later (January 2000).

I used the above fruits to examine fruit weight and percentage seed set for sites WAR1, WAR2, WAR4, WAR5, QT1 and QT3. For the other seven sites, I picked a sample of 10-20 randomly selected fruits from 4-5 trees in the summer of 2000 (range of n=52-96 fruits per site) and used these fruits to examine fruit weight and percentage seed set. Fruits of *E. lucida* dehisce approximately 12 months after the end of flowering (Read 1989). At the time of picking, the fruits in the present study were beginning to dry out but had not yet started to split. All fruits were left in open plastic containers at room temperature for around six months, by which time all seed capsules that were capable of opening had done so. Prior to removing seeds, all fruits were weighed to the nearest 0.001 g. The number of developed seeds (fully expanded) and undeveloped seeds (small or only slightly expanded) were then counted for all those fruits in which all the

seed capsules had dehisced. A portion of fruits failed to fully dehisce, and seed counts for these fruits were not obtained. I assumed that the total number of ovules present in the ovary of a flower equaled the sum of the developed and undeveloped seeds present in the fruit. Percentage seed set was calculated as (developed seeds /[developed + undeveloped seeds])*100.

Statistical analyses

I used a hierarchical ANOVA to test for an effect of apiary (fixed factor) on the percentage of white anthers (data arcsine transformed; location and tree as random factors); on the number of grains per stigma-lobe section (data log transformed; location, tree and flower as random factors); on percentage fruit set (data arcsine transformed); on percentage fruit dehisced (data arcsine transformed); on fruit weight (data square-root transformed; location and tree as random factors); and on percentage seed set (no transformation required; location and tree as random factors). For the percentage of white anthers in flowers of known age, I sampled the same flowers on different days (i.e. repeated measures). However, I did not record flowers individually, making it impossible to use a repeated measures analysis on the raw data. Therefore I pooled the data for individual trees and used a repeated measures ANOVA on the mean values for individual trees. Data for Day 10 were excluded from this analysis due to the very large number of zeros.

Data presented as means±se.

Results

Pollen removal

The mean percentage of anthers carrying pollen varied substantially, ranging from < 1% at WAR2 to > 50% at WAR6 (Fig. 9.1). There was a significantly greater proportion of anthers bearing pollen at control sites compared to apiary sites ($F_{1,1353}$=9.39, P<0.005; Fig. 9.1). Overall, the mean percentage of pollen-bearing anthers was 4.46±0.40% (n=746) and 22.39±1.08% (n=714) for apiary and control sites, respectively.

Changes in the percentage of anthers bearing pollen with flower age was similar for all sites, with an initial increase in the percentage of pollen-bearing anthers over the first 3-5 days as anthers dehisced, followed by a decline as pollen was removed by insects (Fig. 9.2a,b,c). The apparent increase in the percentage of anthers bearing pollen between Days 7-10 at WAR6 (Fig. 9.2a) is presumably the result of recording error. For Days 3,5 and 7, there was a significant effect of apiary on the percentage of anthers bearing pollen ($F_{1,74}$=29.22, P<0.001) and a significant effect of day ($F_{2,74}$=17.61, P<0.001),

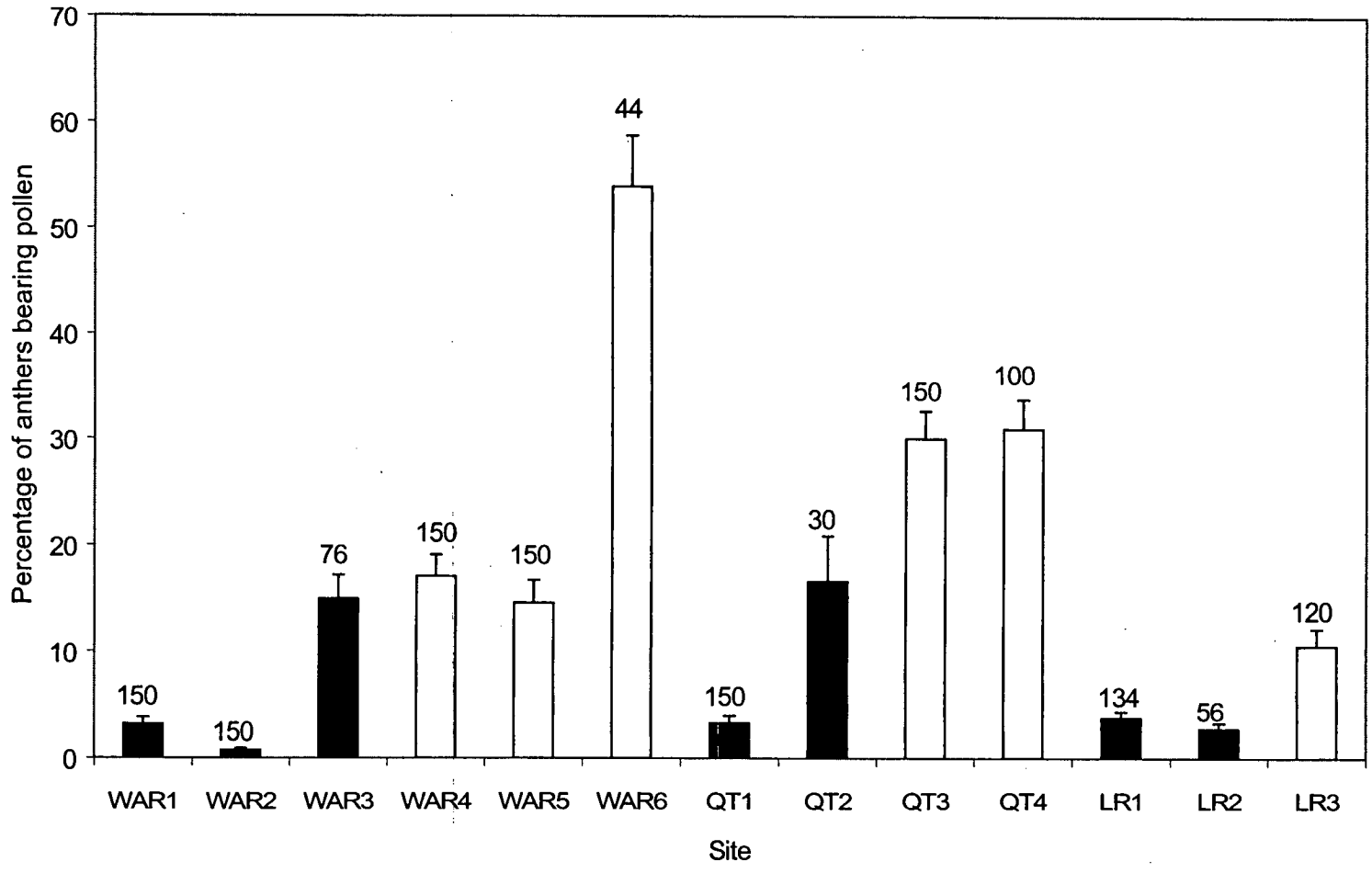

Fig. 9.1. Mean percentage of anthers bearing pollen in *E. lucida* flowers at apiary and control sites. Filled bars are apiary sites, open bars are control sites. Sample sizes given at top of bars. Error bars are standard errors.

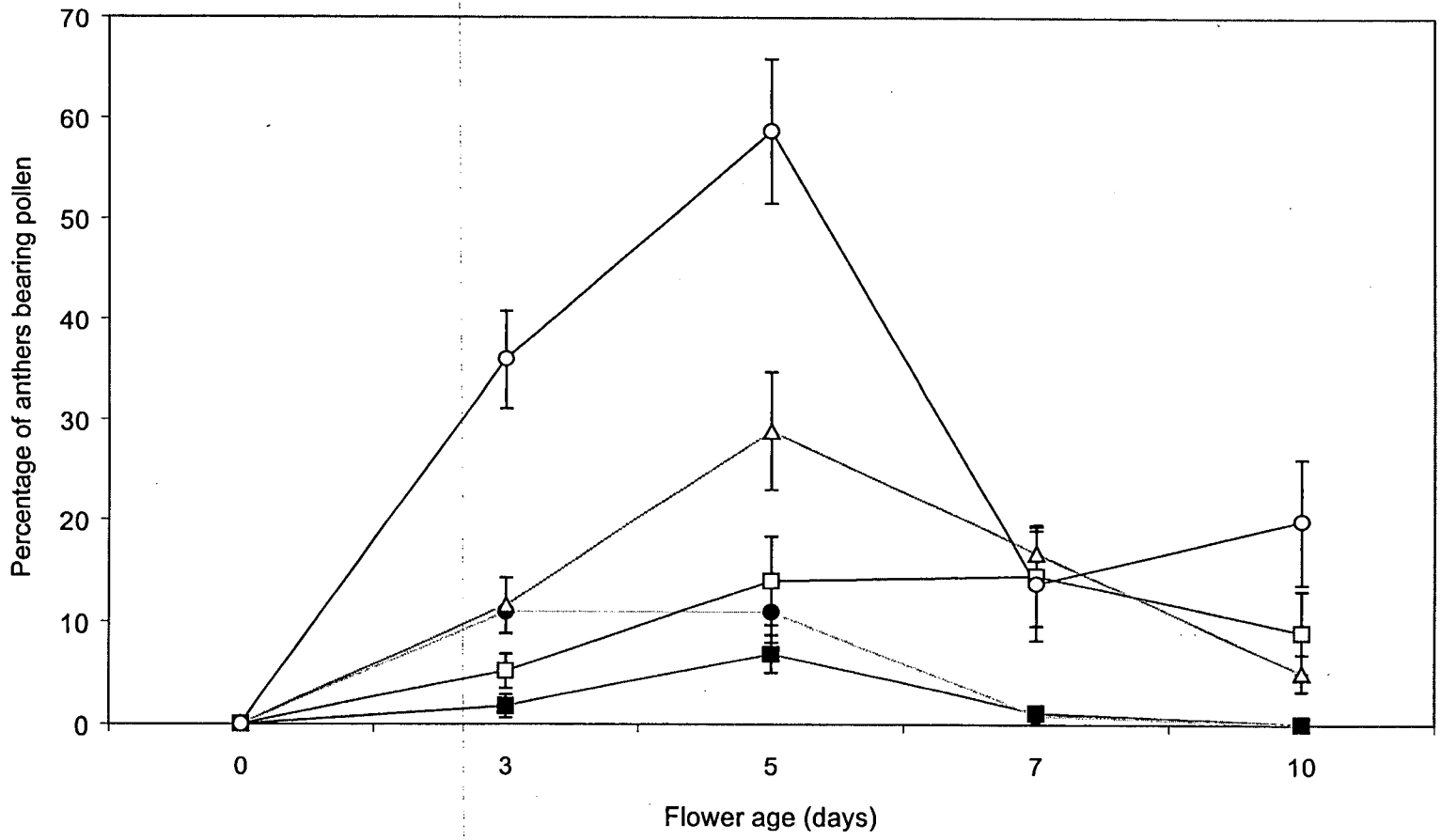

Fig. 9.2a. Changes in the percentage of anthers bearing pollen with flower age at the Waratah sites. Filled symbols are apiary sites, open symbols are control sites. Filled square – WAR2; filled circle – WAR3; open triangle – WAR4; open square WAR5; open circle – WAR6. Error bars are standard errors.

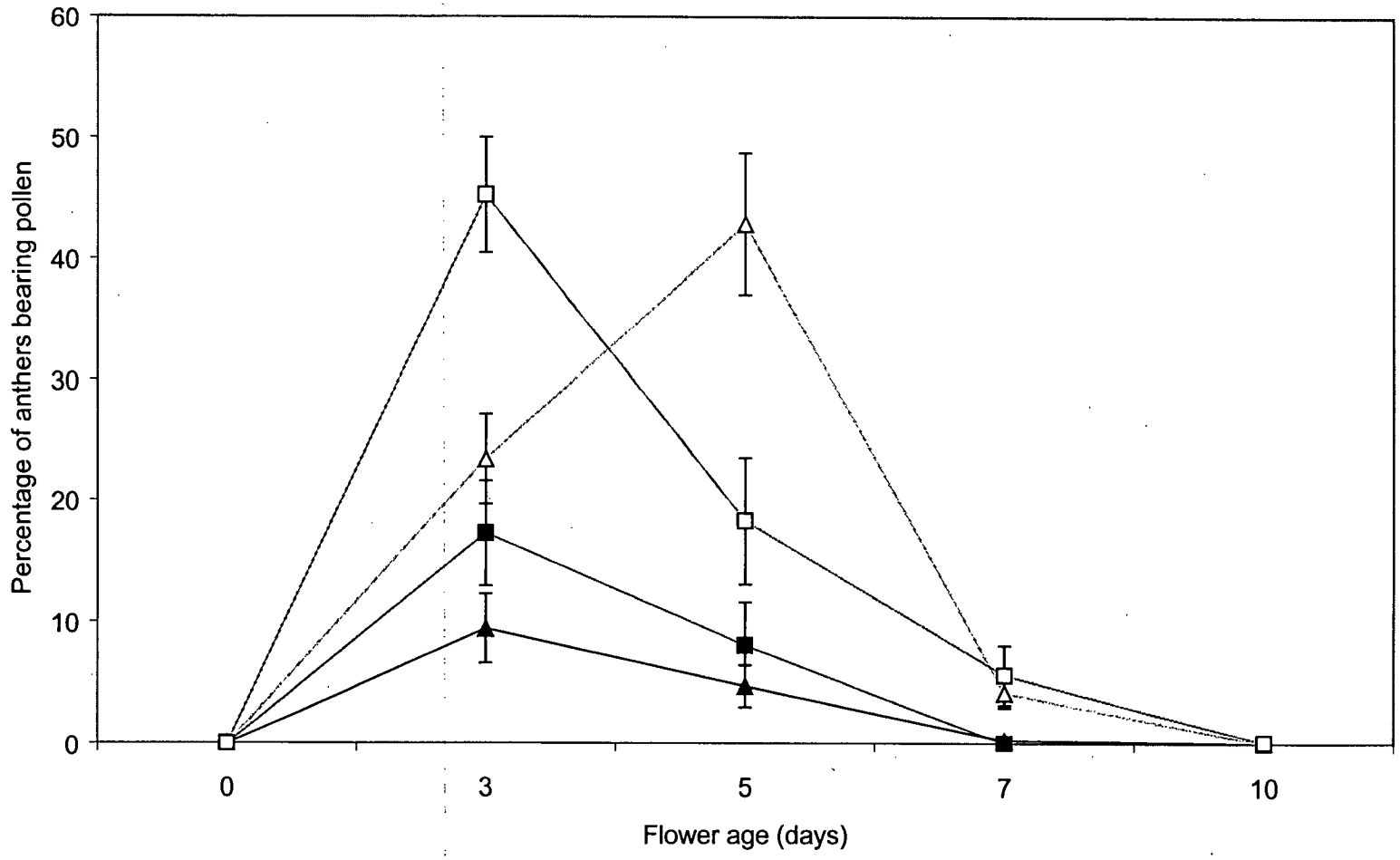

Fig. 9.2b. Changes in the percentage of anthers bearing pollen with flower age at the Queenstown sites. Filled symbols are apiary sites, open symbols are control sites. Filled triangle – QT1; filled square – QT2; open triangle – QT3; open square – QT4. Error bars are standard errors.

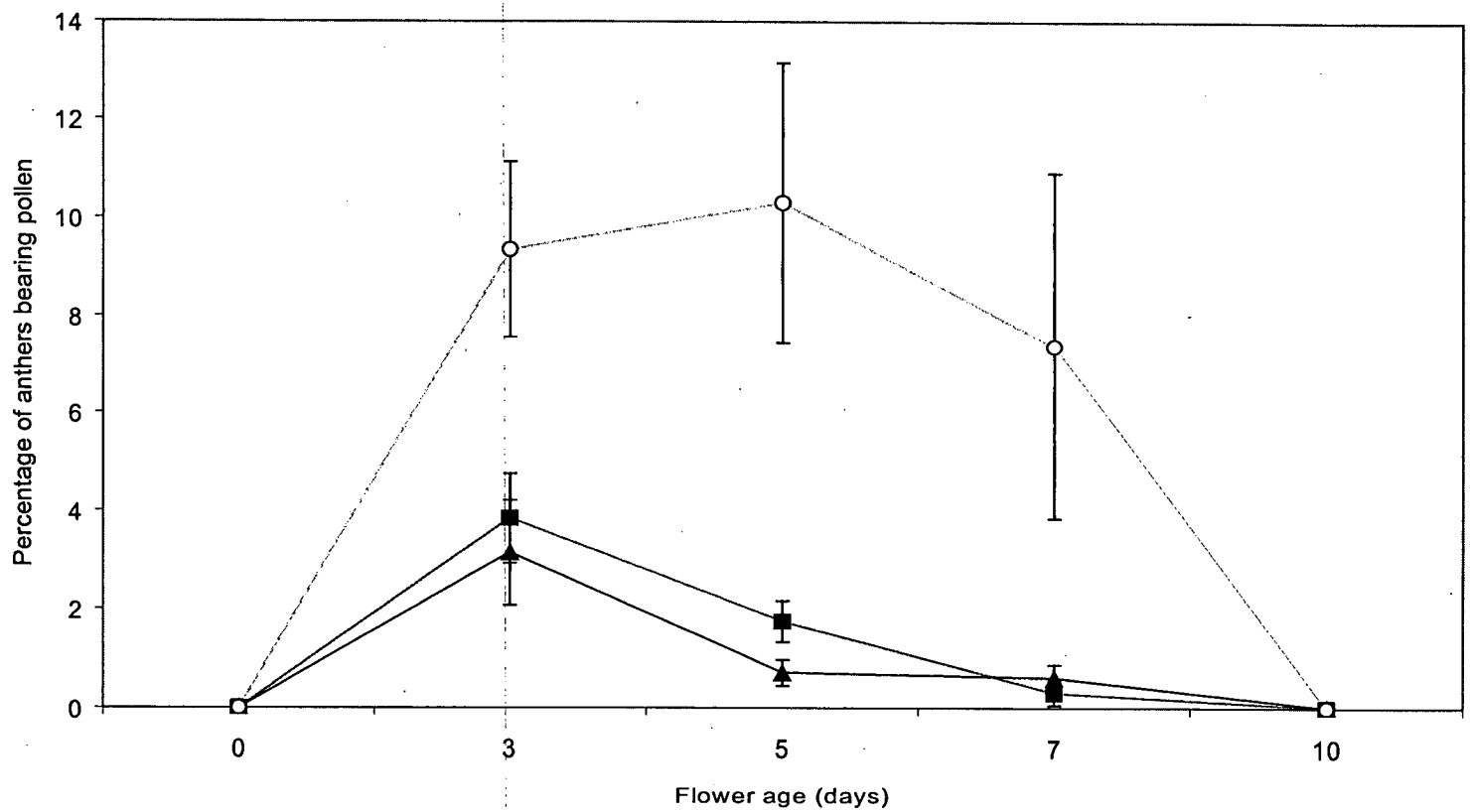

Fig. 9.2c. Changes in the percentage of anthers bearing pollen with flower age at the Link Road Sites. Filled symbols are apiary sites, open symbols are control sites. Filled triangle – LR1; filled square – LR2; open circle – LR3. Error bars are standard errors.

but no interaction between factors ($F_{2,74}$=1.38, P>0.2). By Day 10, all pollen had been removed from flowers at all apiary sites, while 10-day old flowers at control sites frequently contained pollen, particularly at the Waratah sites (Fig. 9.2a,b,c). There was generally less pollen in flowers at the Link Road sites compared to the other locations, although the difference between apiary and control sites was still very marked (Fig. 9.2c).

Pollen deposition

The number of pollen grains recorded on the distal section of stigma lobes varied substantially, ranging from < 4 grains per section at QT2 and QT4 to > 40 grains per section at WAR6 (Fig. 9.3). There was no effect of apiary on the number of pollen grains ($F_{1,664}$=1.59, P>0.2). Overall, there were 12.47±1.41 (n=541) grains per section and 15.12±1.61 (n=357) grains per section at apiary and control sites, respectively.

Fruit set, seed set and fruit weight

Of the 900 flowers tagged in 1999 to estimate fruit set, 784 (87.1%) tags were still present 12 months later. Fruit set at the six sites examined varied from *ca.* 40% at WAR4 and WAR5 to > 90% at QT1 (Fig. 9.4). There was a tendency for fruit set to be lower at the control sites compared to apiary sites. This difference was significant ($F_{1,24}$=16.68, P<0.001). Overall, mean fruit set was 74.27±5.91% and 46.58±5.13% at apiary and control sites, respectively. Similarly, the percentage of fruit which fully dehisced varied substantially between sites, ranging from *ca.* 15% at WAR5 to > 85% at LR1 (Fig. 9.5). There was no effect of apiary on the percentage of fruit which fully dehisced ($F_{1,48}$=3.12, P>0.05). Overall, 59.26±5.23% (n=34) and 55.68±5.60% (n=38) of fruit fully dehisced at apiary and control sites, respectively. Mean fruit weight was relatively consistent between sites, ranging from 0.065±0.004 g at WAR5 to 0.130±0.006 g at LR2 (Fig. 9.6). There was no effect of apiary on fruit weight ($F_{1,832}$=1.58, P>0.2). Overall, mean fruit weight was 0.091±0.002 g and 0.081±0.002 g at apiary and control sites, respectively. Similarly, the mean seed set of fully dehisced fruit was relatively consistent between sites, ranging from 30.01±3.34% at WAR4 to 45.81±1.98% at QT1 (Fig. 9.7). There was no effect of apiary on percentage seed set per fruit ($F_{1,422}$=1.41, P>0.2). Overall, mean seed set was 35.82±0.96% and 28.53±1.31% at apiary and control sites, respectively.

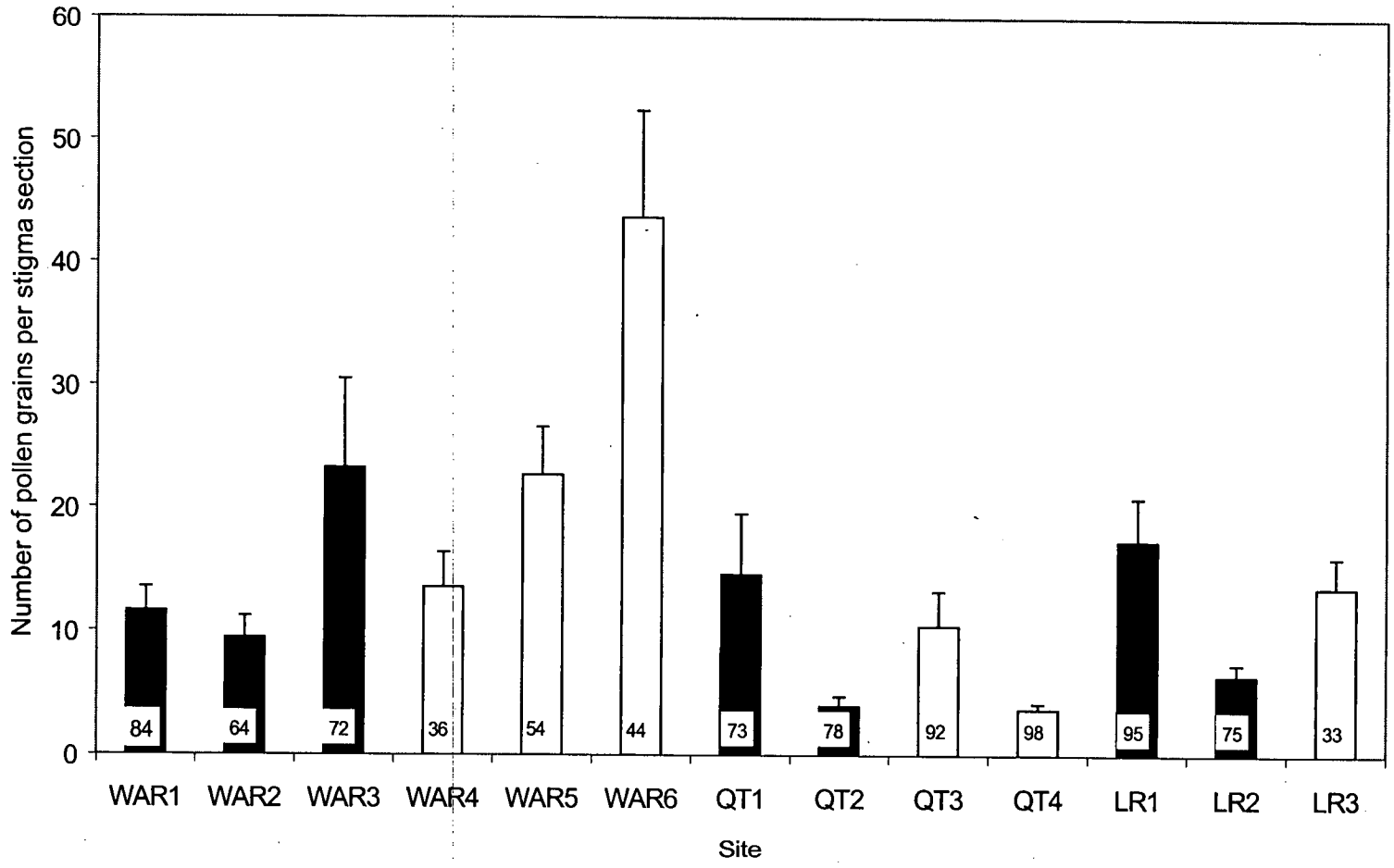

Fig. 9.3. Mean number of pollen grains deposited on the distil 448 μm-section of stigma lobes in *E. lucida* flowers at apiary and control sites. Filled bars are apiary sites, open bars are control sites. Sample sizes given at bottom of bars. Error bars are standard errors.

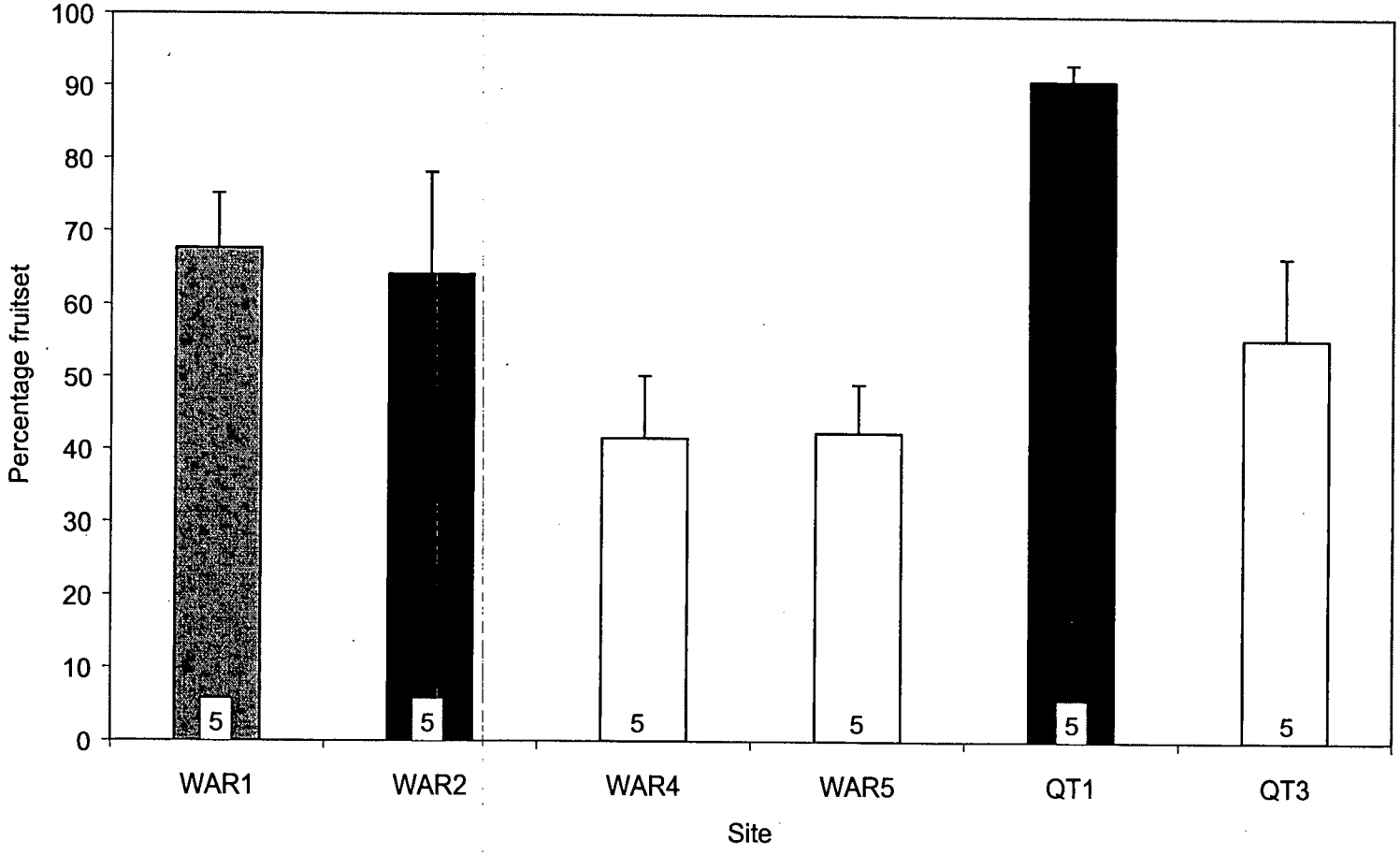

Fig. 9.4. Mean percentage fruit set per tree at apiary and control sites. Filled bars are apiary sites, open bars are control sites. n = 5 trees for all sites. Error bars are standard errors

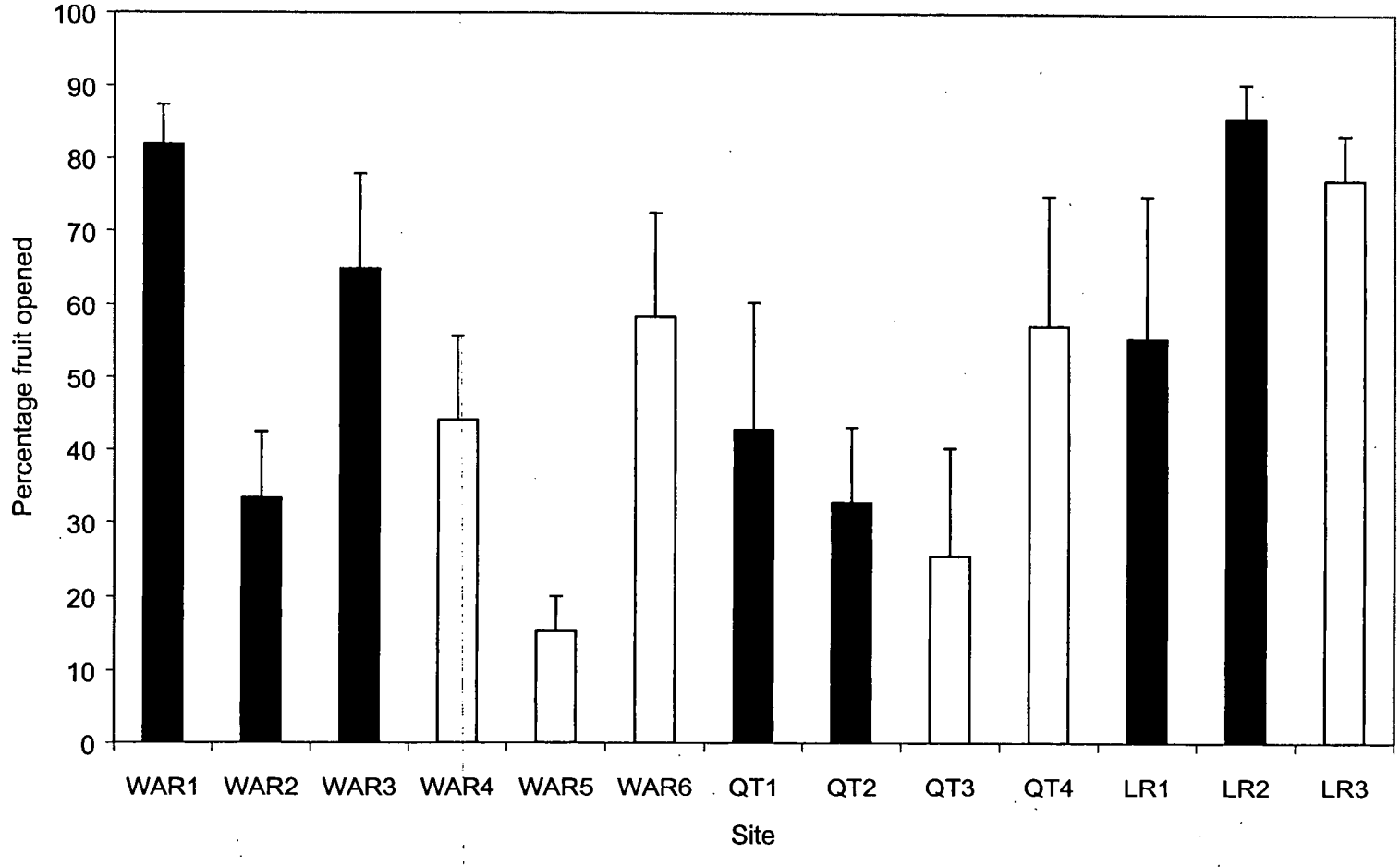

Fig. 9.5. Mean percentage fruit which fully dehisced at apiary and control sites. Filled bars are apiary sites, open bars are control sites. n = 4-5 trees for all sites. Error bars are standard errors.

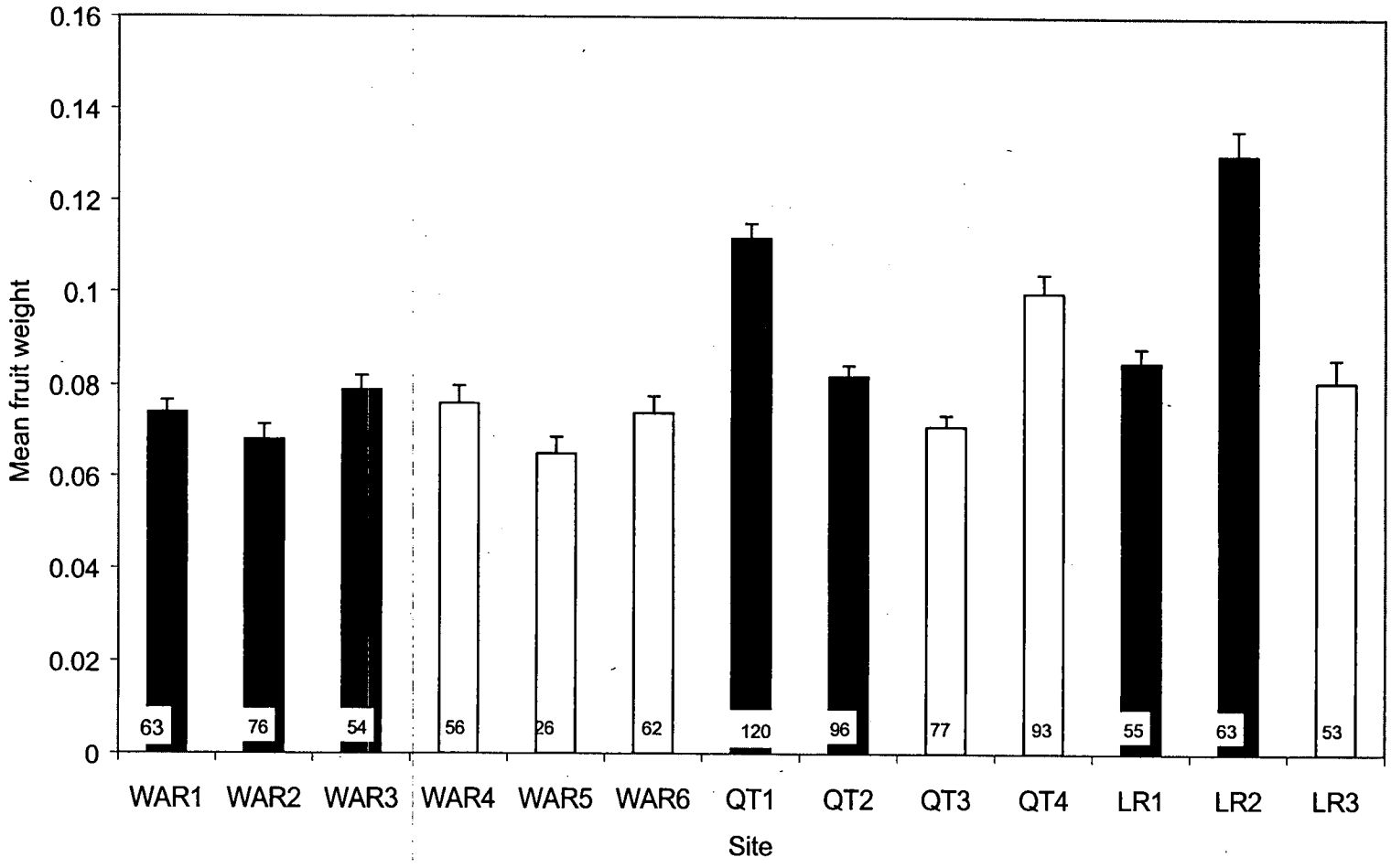

Fig. 9.6. Mean fruit weight at apiary and control sites. Filled bars are apiary sites, open bars are control sites. Sample sizes given at bottom of bars. Error bars are standard errors.

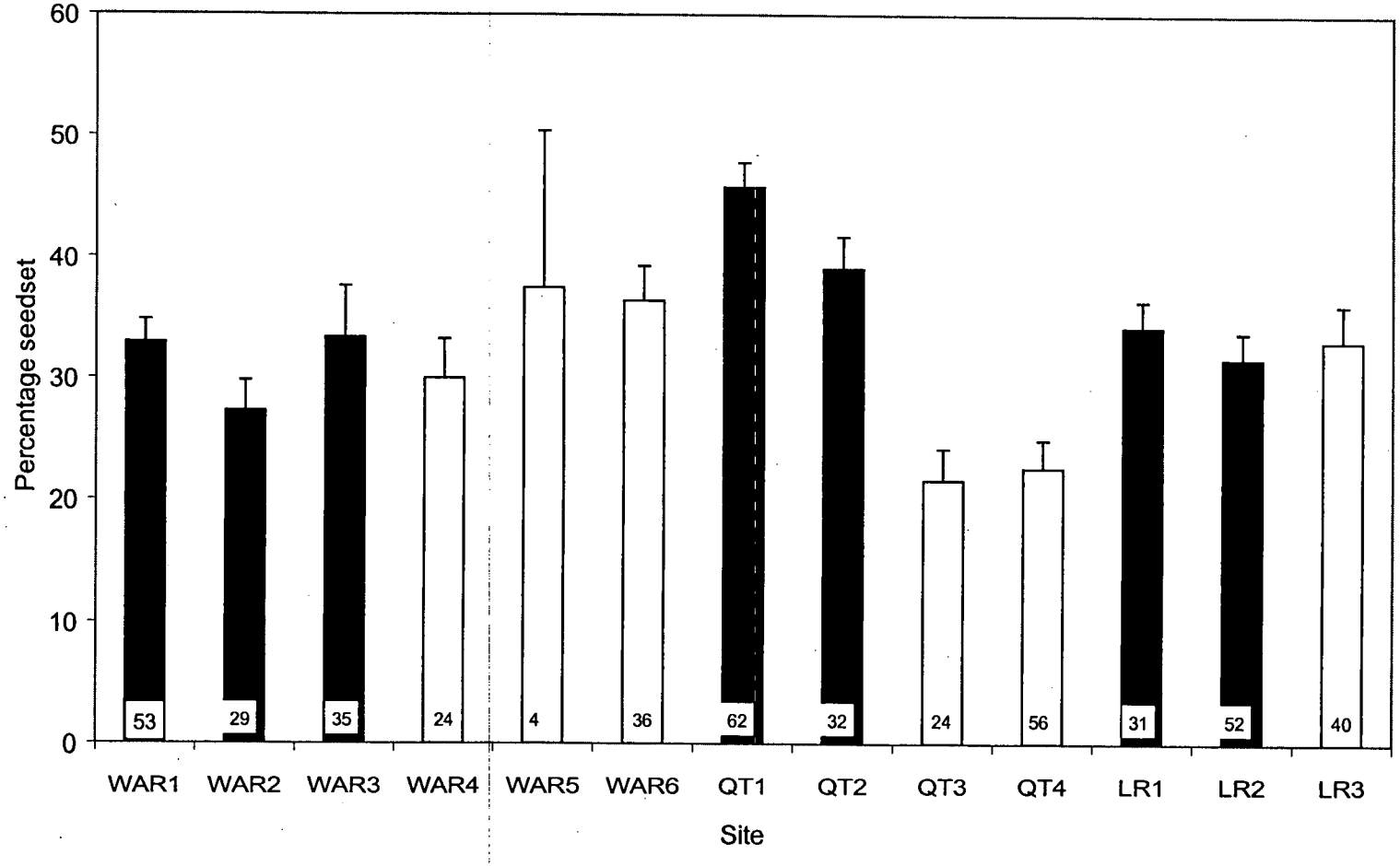

Fig. 9.7. Mean percentage seed set at apiary and control sites. Filled bars are apiary sites, open bars are control sites. Sample sizes given at bottom of bars. Error bars are standard errors.

Discussion

Placement of commercial loads of honeybee hives into native cool temperate rainforest resulted in an increase in honeybee visitation rates to *E. lucida* flowers (see Chapter 8). The majority of these honeybee visits were for nectar, with < 10% of visits involving an active raking of pollen onto the bee's body (Chapter 8). Very large amounts of pollen may be removed during a single honeybee visit to a flower, particularly where honeybees are the first arrivals at flowers and preempt native foragers. For example, Paton (1993) found that where honeybees were the first visitor to the protandrous flowers of *Correa reflexa,* they removed or dislodged 87% of accumulated pollen, compared to honeyeaters which removed only 34-54% of pollen during an initial visit to a flower. *E. lucida* flowers contained negligible amounts of pollen immediately after being visited by a honeybee. Presumably, the majority of pollen present within a flower is picked up by honeybees, both during a pollen-collecting visit as well as passively during nectar collection as the bee moves its body over the mass of anthers and probes downwards to the nectaries at the base of the flower (see Chapter 3).

However, the fate of the pollen may differ for the two types of behaviour. At least a portion of the pollen picked up by pollen-collecting honeybees is raked into the corbiculae and is presumably unavailable for transfer to anther flower, although substantial amounts of residual pollen typically remain on the honeybee's body for pollination purposes (Thomson 1986). In contrast, much of the pollen picked up by nectar collecting bees may remain on the general body surface, and nectar collecting honeybees may therefore carry very large amounts of transferable pollen (see Chapter 3). This assumes that nectar-collecting honeybees do not rake the passively collected pollen into the corbiculae during flight. Certainly, honeybees returning to the hive and carrying large loads of *E. lucida* nectar (see Chapter 3) did not carry noticeable pollen loads.

Data from the present study indicate that hive honeybees in the vicinity of an apiary, although visiting flowers for nectar rather than for active pollen collection, do effect a rapid removal of pollen from dehiscing anthers (Fig. 9.2a,b,c), and cause an overall depression in the 'standing crop' of available pollen in flowers (Fig. 9.1).This more rapid removal of *E. lucida* pollen and the resulting reduction in the standing crop of pollen available in flowers could impact on the native plant-pollinator system via three different pathways. (a) By reducing available pollen as food for native insects, resulting in a reduction in the abundance of native pollinators or their activity at flowers. (b) By reducing the pool of available pollen to be picked up and transferred by legitimate pollinators. And (c), by altering the dynamics of pollen presentation and floral

development, resulting in a change in the relative rates of self and cross pollination.

E. lucida flowers receive visits from a wide range of native insects including flies, beetles, native bees and wasps, and butterflies and moths (see Chapter 3). Of these, the large flies appear to be the principal pollinators based on visitation rates, pollen loads and feeding behaviour at flowers, although the larger beetles may also be important at certain times and locations. Of the larger flies, only the Syrphidae were observed feeding on pollen. Beetles were mainly pollen feeders, native bees took both nectar and pollen, while butterflies and moths were nectarivorous (Chapter 3). Despite a significant reduction in available nectar and pollen (the present study) around apiary sites, there was no evidence of an effect of hives on the abundance or visitation rates by the principal pollinators (large dipterans) or total native invertebrates (see Chapter 8). The absence of any impact of reduced nectar levels on (the principally nectivorous) large dipterans was attributed to the apparent surplus of nectar produced by *E. lucida* flowers. Similarly, although the standing crop of pollen in male flowers was significantly reduced around apiaries (*ca.* 5% of anthers bearing pollen) compared to control sites (*ca.* 22% of anthers bearing pollen), there may also have been sufficient pollen even in the presence of hive bees to support the native polleniforous fauna (*cf.* Paton 1999).

However, a 17% reduction in the standing crop of pollen available in flowers may represent a significant depletion in the pool of pollen available for pick-up by pollinators. Pyke (1990) has pointed out that the removal of pollen from flowers by honeybees could lead to a reduction in the amounts of pollen picked up on the bodies of legitimate pollinators, and consequently to a diminished native pollination service. This phenomenon was observed by Paton (1993; also 1996, 1997) in flowers of *Correa reflexa*. Honeybees are typically the first visitors to *C. reflexa* flowers, and flowers which receive several honeybee visits before the first visit by a honeyeater retain very little pollen for the native pollinator to collect (Paton 1993). Field trials indicated that a 90% reduction in available pollen in source flowers led to an 83% decline in the number of pollen grains received by virgin *C. reflexa* flowers (Paton 1993). Gross and Mackay (1998) observed a related phenomenon in the pioneer shrub *Melastoma affine*. *M. affine* is pollinated by native bees, but received increasing numbers of honeybee visits with the introduction of commercial hives to the study site. Honeybees were found to be poor pollinators of *M. affine* flowers relative to native bees. In addition, honeybees actively stripped *M. affine* stigmas of previously deposited grains, leading to a significant reduction in fruit and seed set in the presence of hive bees (Gross an Mackay 1998).

Despite a significant reduction in the pool of available pollen for pickup by native pollinators, I found no evidence for an effect of hive bees on the number of pollen grains deposited on stigmas. There was a tendency for fruit set to be higher near apiaries, although this result was derived from only six of the study sites. In contrast, there was no effect of apiaries on fruit dehiscence, fruit weight or seed set. Clearly, if hive honeybees were disrupting the native pollinator service by reducing the pool of available pollen, they apparently also provided a compensatory service leading to little net change in the reproductive performance of *E. lucida*.

Honeybees have been found to be effective pollinators of some *Banksia* species (Whelan and Burbidge 1980; Paton and Turner 1985; Vaughton 1992; Paton 1999) but not others (Whelan and Burbidge 1980; Collins and Spice 1986; Ramsey 1988), while honeybees apear to be ineffective pollinators of a range of other native plants including *Callistemon rugulosus* (Paton 1993, 1996, 1997), *Correa reflexa* (Paton 1993, 1996, 1997), *M. affine* (Gross and Mackay 1998) and *Calothamnus quadrifidus* (Collins *et al.* 1984). Honeybees carry large amounts of *E. lucida* pollen on their ventral surface, make frequent contact with stigmas, and deposit around five *E. lucida* pollen grains per visit (see Chapter 3). Honeybees therefore appear to be 'effective' pollinators in the sense of regularly depositing pollen on conspecific stigmas. However, the relative proportion of self and cross pollen deposited by honeybees may differ from that deposited by native pollinators (Paton 1993, 1996; Aizen and Fiensinger 1994b).

Self pollen deposited onto stigmas may be either from within-flower (autogamous) or within-tree (geitonogamous) transfer (Lloyd and Schoen 1992). Autogamous deposition of self pollen onto the stigma of a flower may occur autonomously (i.e. without the aid of a vector), or occur through the agency of a pollinator ('facilitated' selfing; Lloyd and Schoen 1992). The extent of autogamous selfing in a flower will depend to a large extent on the proximity of anthers and stigmas, and on the degree of overlap in pollen presentation and stigma receptivity within a flower. In contrast, levels of geitonogamy depend on the relative proportion of self and cross pollen carried by pollinators, the number of flowers visited on a plant during a visitation sequence, and the degree of carryover of self pollen from one flower to the next (Robertson 1992; de Jong *et al.* 1993).

Honeybees could potentially influence the reproductive success of a plant by altering the proportions of self and cross pollen deposited via changes in the rate of autogamous and/or geitonogamous selfing. The former may be particularly likely in protandrous species where the duration of the initial male

phase, and therefore the degree of overlap in pollen presentation and stigma receptivity, depends on the rate at which pollen is dislodged from anthers (Vaughton and Ramsey 1991; see Chapters 2 and 4). In partially protandrous flowers in which the anthers and stigma are in close proximity, any increase in the overlap between pollen presentation and stigma receptivity should result in an increase in the potential for autogamous selfing (Chapter 4).

E. lucida flowers are protandrous and weakly herkogamous. Anthers dehisce over the first 4-5 days of anthesis, with the released pollen being steadily removed during multiple visits by insects (see Chapter 2). Typically, when visitors are frequent, the majority of pollen is removed by the commencement of stigma receptivity at around seven days of age (i.e. dichogamy is close to complete). However, at the other extreme (i.e. in bagged flowers protected from visitors), pollen accumulates on anthers well into the female phase of anthesis, leading to substantial autogamous deposition of self pollen onto receptive stigmas (see Chapter 2). In Chapter 4, I hypothesised that a decrease in visitation rate will be negatively correlated with the degree of overlap in pollen presentation and stigma receptivity, and with the potential for autogamous deposition of self pollen in *E. lucida* flowers.

Results of the present study suggest that the presence of hive bees increases the rate of pollen removal from *E. lucida* flowers during the male phase, and reduces the degree of overlap between pollen presentation and stigma receptivity. At apiary sites, > 99% of pollen was removed from flowers by Day 7 (Fig. 9.2a,b,c). In contrast, flowers at control sites frequently had > 10% of anthers still bearing pollen at the commencement of stigma receptivity around Day 7, with some flowers still carrying appreciable amounts of pollen well into the female phase (Fig. 9.2a,b,c). By increasing the rate of pollen removal and reducing the overlap between pollen and stigma receptivity, hive bees may therefore be reducing the quantity of autogamous self pollen deposited onto *E. lucida* stigmas.

This hypothesis gains some support from a positive and significant relationship between the percentage of anthers still bearing pollen during the female phase (average of the Day 7 and 10 values in Fig. 9.2) and the number of pollen grains on stigmas (Fig. 9.3) ($F_{1,12}=5.56$, $r^2=0.34$, $P<0.05$; Fig. 9.8). That is, sites with a substantial retention of pollen into the female phase (e.g. WAR6; Fig. 9.2a) tended to have more pollen grains deposited on stigmas, presumably through autogamous deposition of self pollen. Vaughton (1992) has described a similar relationship between honeybee visits and the degree of dichogamy in the protandrous *Banksia spinulosa*. In late season when honeybees were active, caged *B. spinulosa* inflorescences (visited by honeybees but not birds) were

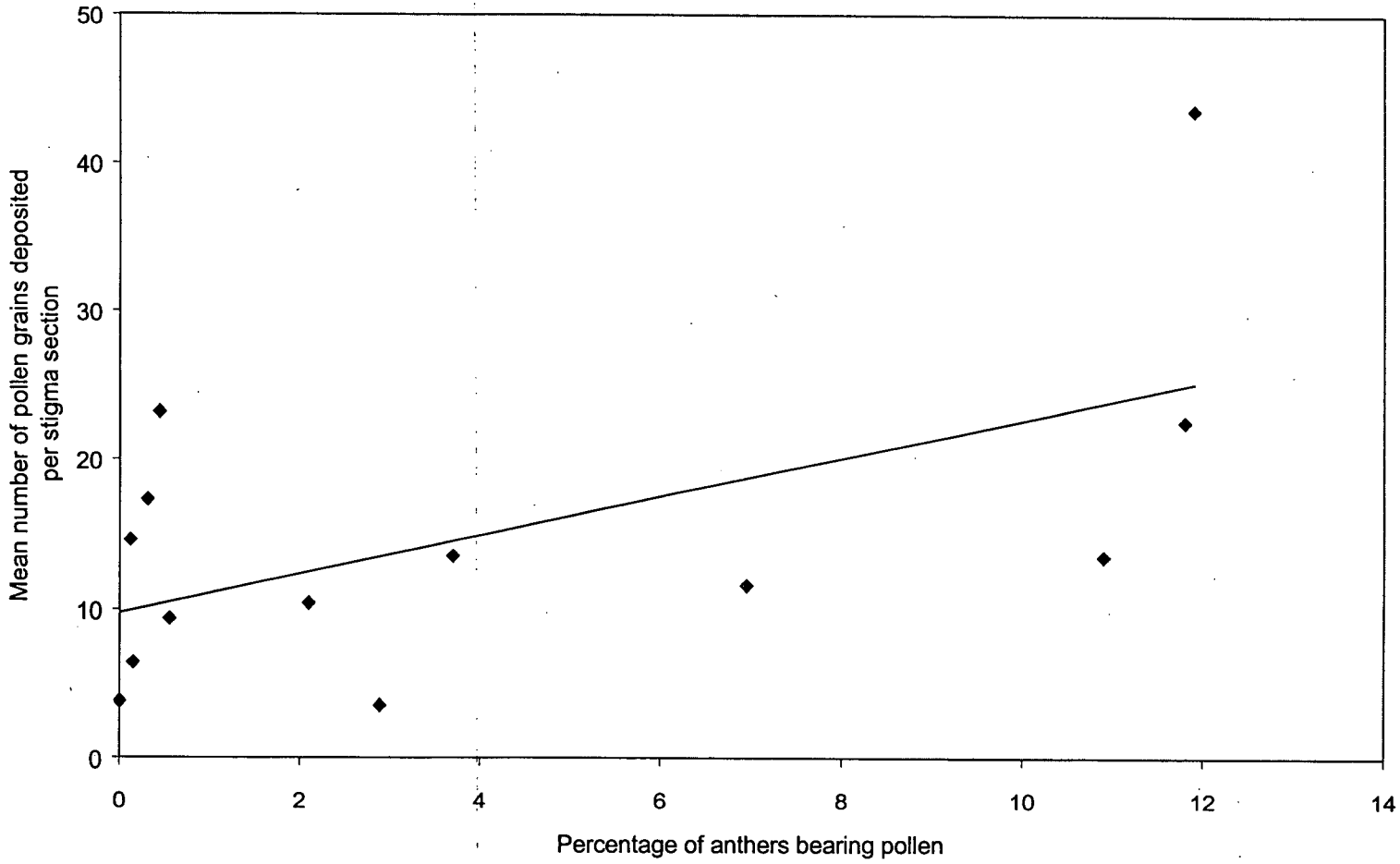

Fig.9.8. Regression of the mean percentage of anthers bearing pollen (average for Day 7 and Day 10 flowers) against the mean number of pollen grains deposited on stigmas for the thirteen sites. Linear regression line is shown.

rapidly depleted of pollen, leading to complete segregation of the male and female phases. In contrast, bagged inflorescences (honeybees and birds excluded) retained the bulk of their pollen well into the period of maximal stigma receptivity, leading to an increased potential for autogamous self pollination of flowers (Vaughton 1992; see also Vaugthon 1988; Vaughton and Ramsey 1991).

E. lucida is partially self-fertile, although selfed flowers set relatively low levels of fruit and seed (Chapter 2). If, as suggested above, there was an increased tendency for self pollen to be autogamously deposited onto stigmas at control sites, such increased selfing should have been accompanied by a decrease in fruit and seed set. Yet flowers at control sites had similar fruit and seed set to apiary sites where autonomous selfing was largely prevented by the rapid removal of pollen by hive bees. This in turn suggests that pollen deposited by hive honeybees at apiary sites included a high proportion of geitonogamous self pollen transferred between flowers of the same tree. Honeybees visit on average around 8-10 flowers on a tree during a visitation sequence and between 2-4 trees during a foraging bout (see Chapter 3), so clearly the potential for honeybee-mediated geitonogamy is very high.

Further work on rates of autogamous selfing in *E. lucida* and pollen carryover and geitonogamous selfing rates by honeybees are clearly needed to test these hypotheses. However, data presented here suggest that the presence of commercial honeybee hives results in little net impact on the female reproductive performance of *E. lucida*. Furthermore, the absence of any effect of hive bees on the number of pollen grains deposited on stigmas or on fruit weight and seed set may be a reflection of high levels of pollen retention and increased levels of autogamous selfing at control sites, and to high rates of geitonogamous pollinations by honeybees.

Chapter 10. Foraging range of hive honeybees during *E. lucida* flowering

Abstract

The foraging range of hive honeybees working *E. lucida* forest was estimated for five apiaries in west and north-west Tasmania. The foraging range was estimated from the total sugar yield for the apiaries using data on nectar production and consumption rates, the number of *E. lucida* flowers per hectare, the number of foraging days, and the proportion of the area around the apiaries which was occupied by forest containing *E. lucida*. The foraging range for the five apiaries ranged from 1.1 to 4.6 km (mean±se = 2.08±0.64 km), although the figure of 4.6 km appeared to be atypical due to the scarcity of *E. lucida* forest around this apiary site (LR2). The mean foraging range for the six apiary sites excluding this anomalous site was 1.45±0.13 km (range 1.1 - 1.7 km). A foraging range of *ca.* 1.5 km is consistent with other reports of the foraging distance of hive bees when resources are freely available, and is in good agreement with the current minimum separation of commercial apiary sites of 3.0 km.

Introduction

The mean foraging distance of honeybees from their hives varies with foraging conditions (e.g. wind, temperature, rainfall), and with the nature of the forage resource (Wenner 1992). While bees are capable of foraging up to 14 km from the hive when resources are scarce, the majority of a hive's bees appear to forage within 1-2 km where resources are freely available (Sudgen and Pyke 1991; Wenner 1992; Paton 1996; Manning 1997 and references therein).

Despite the importance of *E. lucida* to commercial honey production in Tasmania, there is little information on the foraging range of hive bees in cool temperate rainforest. Anecdotal evidence from Tasmanian beekeepers suggests that hive bees forage within 3 km of apiary sites when foraging on leatherwood (Ziegler 1993). Accurate information on foraging range of hive bees is important for a number of reasons. Efficient placement of commercial apiaries to maximise honey yields requires information on how far the bees are flying and the influence of topography (Manning 1997), while estimates of honey yield per area of resource can be heavily biased by very small differences in assumed foraging range (Ziegler 1993). The potential pickup of forage contaminants (such as pesticides) and the transfer of bee diseases between apiaries will also be a function of the bees' foraging range.

Foraging distance can be measured directly in the field by marking bees and later retrieving them from the hive. Alternatively, the 'dances' of foragers

can be interpreted by a human observer and provide approximate distance and direction information (e.g. Wenner 1992). Where information is available on nectar levels in the forage species the proportion of this nectar being consumed by hive bees, foraging distance may also be estimated indirectly by back-calculating the area of forage needed to produce a known honey yield. Using data on diurnal nectar production and consumption rates (Chapter 8) and the insect fauna visiting flowers (Chapter 3), I employed the latter method to estimate the foraging range of hive honeybees foraging on *E. lucida* for five of the seven apiary sites.

Methods

Apiary sites

I studied honeybee foraging range at the three Waratah apiaries (WAR1, WAR2 and WAR3) and two at the Link Road apiaries in 2000 (LR1 and LR2). Unfortunately, honey yields were unavailable for the two Queenstown apiaries (QT1 and QT2). See *Study Sites* section for details of sites.

Consumption of E. lucida *nectar by honeybees*

I obtained information on the total daily production and consumption of nectar sugar per *E. lucida* flower and on the proportion of total insect visits to flowers made up by honeybees at the five apiary sites (see Table 8.1, Chapter 8 and Table 3.2, Chapter 3). I assumed that the amount of nectar sugar consumed by honeybees was proportional to their abundance at flowers (*cf.* Paton 1996).

Number of E. lucida *flowers per hectare*

I used the point-quadrat method (Mueller-Dombois and Ellenberg 1974) to estimate the density of forest trees with basal trunk diameter > 10 cm at each of the three study locations. In addition, I estimated the number of flowers per *E. lucida* tree during peak flowering at each location (see Table 10.1 for details of sampling times). For each location, I selected 10-15 flowering trees per site, with trees on the edge of the track or road-side being avoided to reduce the effect of the canopy gap on flower density. Using 8x40 binoculars, I approximated the shape of the exposed canopy of each tree (as a sphere, cone or cylinder), and estimated the dimensions (radius and height) required to calculate the canopy area. In addition, I scanned 3-4 points on the tree canopy and obtained a mean estimate of the number of flowers per square metre, allowing me to calculate the approximate total number of flowers for each tree.

Table 10. 1. Number of *E. lucida* trees/ha and the mean±se number of flowers per *E. lucida* tree during peak flowering at the three apiary locations. Point counts and sample sizes in brackets.

Location	Sampling date	Trees/ha	Flowers/tree
Waratah	early Feb. 1998	378 (20)	5656.8±1177.2 (30)
Queenstown	mid Jan. 2000	918 (15)	4974.1±579.7 (36)
Link Road	late Jan. 2000	399 (20)	3849.9±652.7 (29)

Estimating foraging range from honey yield

I obtained a figure for the total amount of honey removed by the apiarist from the three Waratah and two Link Road apiaries for the years in which flower density and nectar production were determined (Table 10.1). This amount of honey removed by the apiarist is not the total honey produced over the summer, as some honey is left on hives for winter feed and a proportion of the honey harvested by the bees is consumed by the hive over the summer months. Based on estimates provided by the apiarists concerned, I estimated the amount of honey left for winter feed as 20 kg honey per hive, and the amount of honey consumed over the summer as 3 kg of honey per week per hive. This gave a figure for total honey harvested by each apiary over the summer foraging period. I converted this figure to total sugar, assuming honey is 85% sugar and 15 % water (Paton 1996).

From the relevant apiarists I obtained an estimate of the length of the flowering period, and assumed that only 75% of these days would have had suitable weather for foraging. I then used Equation 1 to estimate the total area of *E. lucida* forest required to produce the known sugar yields for each apiary.

Equation 1: Total honey yield (kg sugar) = sugar removed by honeybees (kg/flower/day) * flowers/ha * forage area (ha) * number of foraging days

Given that the rainforest in which *E. lucida* occurs is very patchily distributed, the area of *E. lucida* forest obtained from Equation 1 is considerably less than the actual area covered by foraging honeybees. I used photo-interpretaion (PI) maps (Forestry Tasmania, Hobart, Tasmania) to estimate the proportion of the area surrounding each apiary which was made up of forest containing *E. lucida*. The forest types used by the PI maps include six categories which could potentially include *E. lucida* (Ziegler 1993). I recorded only the two forest types containing the highest quantity of *E. lucida* (low myrtle forest =

'*m*-' and old growth eucalypt forest with a myrtle understorey = '*E.m*-'; Ziegler 1993). I recorded the proportion of the area within a circle of 2 km radius centered on each apiary site which was made up of *m*- and *E.m*- forest, and 'corrected' the figure for foraging area of *E. lucida* forest obtained from Equation 1 to give an estimate of the actual foraging area covered by the hive bees. Assuming a circular foraging area centered on the apiary site, this gave an estimate of the foraging range of the hive bees.

Results and Discussion

The density of *E. lucida* trees was similar at the Waratah and the Link road sites (378 and 399 trees/ha), but substantially higher at the Queenstown sites (918 trees/ha), while the number of flowers per tree was relatively consistent between areas (Table 10.1). Ettershank and Ettershank (1992) estimated the number of flowers per *E. lucida* tree at a site in south-west Tasmania (my MAY site) by approximating the tree shape. Their estimates were slightly lower than in the present study, ranging from *ca.* 100 –3000 flowers/tree. Ettershank and Ettershank (1992) also estimated the density of flowering *E. lucida* stems using a quadrat method at MAY. Once again, their estimates (range of 80 – 480 flowering stems/ha, mean = 275±102, n = 4) were similar although somewhat lower than the estimates in the present study (Table 10.1).

The total sugar yield for Waratah and Link Road apiaries varied substantially, ranging from 1.8 – 8.2 tonnes of sugar, and was not obviously related to the number of hives present (Table 10.2). The amount of sugar produced by *E. lucida* flowers also varied substantially between apiary sites, ranging from < 1 to > 4 mg sugar flower per day (Table 10.2). Honeybees were an important component of the flower visiting fauna at all sites, making up between 31.9 – 79.8% of the total insect visits to flowers, and were presumed to be consuming nectar in proportion to their frequency of visits (Table 10.2). The number of days on which honeybees were estimated to have foraged was relatively limited, ranging from only 15 – 21 days (Table 10.2). Based on these data, the predicted area of *E. lucida* forest utilised by the hive bees ranged from 129.7 – 326.9 ha (Table 10.2). The proportion of the area around the apiaries made up of *E. lucida* forest was relatively consistent for four of the five apiary sites (25 – 37 %), and very low (5 %) for the remaining apiary site (LR2) (Table 10.2). Similarly, the total honeybee foraging area and foraging range were relatively consistent for the first four sites (405.4 – 952.4 ha and 1.1 – 1.7 km, respectively), and relatively very large for LR2 (6538 ha and 4.6 km) (Table 10.2). The LR2 site appears to be exceptional in the scarcity of *E. lucida* forest in the vicinity of the apiary, and in the distance traveled by the hive bees to

Table 10.2. Estimated foraging range of hive bees based on total sugar yield from five apiaries. Calculations assume a circular foraging area centered on apiary site. Total sugar yields include total honey removed by apiarist plus honey left on hives for winter feed (20 kg/hive) plus honey consumed by bees during summer (3 kg honey/hive/week). Consumption values from Table 8.1, Chapter 8. Honeybee visits as a percentage of total flower visits from Table 3.2, Chapter 3. Number of flowers per hectare from Table 10.1. Number of foraging days are 75% of total flowering period (supplied by apiarists). The percentage of foraging area occupied by *E. lucida* forest was obtained from photo-interpretation (PI) maps, Forestry Tasmania.

Site	Hives	Sugar (t)	Cons (mg/fl)	Hb visits (%)	Hb Cons (mg/fl)	fl/ha (10^6)	Days	*E.l.* area	*E. l.* (%)	Tot. area (ha)	Range (km)
WAR1	45	3.5	1.10	59.9	0.63	2.14	21	129.7	32	405.4	1.1
WAR2	60	1.8	1.18	31.9	0.38	2.14	15	144.3	25	577.1	1.4
WAR3	88	6.3	1.26	47.1	0.59	2.14	15	333.3	35	952.4	1.7
LR1	93	8.2	1.10	79.8	0.88	1.54	20	302.9	37	818.7	1.6
LR2	93	4.7	0.99	55.3	0.55	1.54	17	326.9	5	6538.0	4.6

access a suitable area of forest. Furthermore, the honey yield for this site was considered to be marginal for continued commercial operation (R. Charles, apiarist, personal communication). For all apiary sites, the mean predicted foraging range for hive bees was 2.08±0.64 km (n=5). If the LR2 site is excluded from the calculation, the mean predicted foraging range was 1.45±0.13 km (n=4).

A foraging range of *ca.* 1.5 km for honeybees working *E. lucida* forest is consistent with a number of other studies suggesting honeybees forage within 1-2 km of the hive where resources are freely available (Sudgen and Pyke 1991; Wenner 1992; Paton 1996; Manning 1997 and references therein). Further support for a foraging range of < 2 km comes from my comparison of apiary and control sites (see Chapters 8 and 9). The control sites ranged from 2-5 km from the nearest apiary site (see *Study Sites* section and maps). During intensive monitoring of insect visitors to *E. lucida* flowers, very few golden coloured (presumed hive) honeybees were observed at the two control sites (WAR6 and QT4) which were approximately 2 km distant from an apiary (n=2 golden-coloured honeybees recorded at both WAR6 and QT4, respectively, during four days of observation at each site).

A foraging range of 1.5 km is also in good agreement with the current consensus among commercial apiarists and the Tasmanian Government regulatory body for a 3 km minimum separation between commercial apiary sites (Ziegler 1993). However, honeybee foraging distance and direction may be influenced by topography, with hive bees apparently reluctant to travel over ranges (Ziegler 1993; C. Parker personal communication). The apiary sites in the present study were in terrain ranging from relatively undulating (the Link Road sites) to moderately rugged (the Waratah and Queenstown sites). In areas of more broken topography where hive bees are more heavily restricted to gully lines, it may be possible to place apiary sites in closer proximity than the current standard of 3 km without competition occurring between adjacent apiary sites.

Chapter 11. Honey or Chips: putting a value on a leatherwood tree

The harvesting of honey is an important industry in Australia, currently worth around $49 million per annum (Gibbs and Muirhead 1998). Of this total honey production, approximately 80% (or 30 000 tonnes per year) is derived from native species (Gibbs and Muirhead 1998). Commercial honey production in Tasmania makes up *ca.* 2.0% of the national total, and is worth around $1.5 million a year (Gibbs and Muirhead 1998).

The harvesting of honey from native forests to some extent occupies a middle ground in debates on the sustainable use of a natural forest resource. On the one hand, there is the view that honeybees are a feral species and therefore inherently undesirable and potentially harmful to indigenous species and natural systems (Pyke 190; New 1997). On the other hand, however, honey production is frequently viewed as an environmentally friendly and sustainable industry (e.g. Manning 1997), particularly in comparison with other more destructive forest industries such as timber and pulp-wood production.

In Tasmania, a large portion (*ca.* 70%) of the state's commercial honey production is made up of leatherwood honey derived from nectar of the the native Tasmanian leatherwood tree, *E. lucida* (Ziegler 1993). Tasmania also has a very active forestry industry, including the production of both hardwood timber and pulp from native forests. A significant proportion of clear-felling in Tasmania's native forests occurs in 'mixed' forest with an over-canopy of scattered large eucalypts above a mature understory of rainforest species, including *E. lucida* (Ziegler 1993). There is therefore the potential for both a real and for a perceived conflict between forestry practices which remove *E. lucida*-rich forest though clear-felling and the use of such forests for the on-going production of honey.

As part of my three year investigation into the pollination ecology of *E. lucida* and the potential impacts of hive honeybees, I gathered data on nectar production and consumption in flowers (Chapters 8) and honeybee visitation rates for seven apiary sites in western and north western Tasmania (Chapter 3), and used these data to estimate the foraging area of hive bees at five of these apiaries (Chapter 10). In this chapter, I use the known honey yields and forage areas for these five apiaries to calculate the honey yield per hectare of forest, and convert these yields to the total honey value per hectare per year based on the current farm-gate value for leatherwood honey. I then attempt to compare the value of *E. lucida* forest for honey production over the entire 'lifetime' of the forest (approximated as the life-span of a *E. lucida* tree), with the value of

the same forest for wood production (initial clear-felling followed by hardwood plantation).

The honey yield from the forest surrounding the five apiaries ranged from 9.2 – 23.1 kg leatherwood honey per hectare (mean = $15.94), corresponding to a farm-gate return of between $18.35 – $46.22 per hectare of forest (mean = $31.90) (Table 11.1). *E. lucida* trees can live up to 350 years of age. Flowering begins at around 100 years of age in trees growing in un-disturbed forest (Ziegler 1993), although flowering can begin as early as 10 years of age in trees grown in open conditions (Neyland and Hickey 1990). Therefore, the flowering life of a *E. lucida* tree in mature forest is estimated at 250 years. Assuming a 'forest' life-span equivalent to that of individual *E. lucida* trees, then the life-time returns from *E. lucida* forest through leatherwood honey production ranges between $4588 – $11555 per hectare of forest (mean = $7974) based on the per hectare production figures in Table 1. However, the lowest return figure ($18.35) occurred at an apiary (LR2; Table 11.1) which was considered by the apiarist to be marginal for commercial honey production (R. Charles apiarist, personal communication). If this figure is excluded, the mean return from honey production was $35.29 per hectare, or *ca.* $8800 per hectare over 250 years.

Table 11.1. Total honey yields for seven leatherwood-honey apiary sites, showing the estimated area of *E. lucida* forest used for foraging (from Table 10.2, Chapter 10), the honey yield per hectare, and the farm-gate returns to apiarist (assuming a farm-gate price of $2.00/kg honey).

Site	Yield (kg honey)	*E. lucida* forest (ha)	kg honey/ha	Returns/ha
WAR1	2700	129.7	20.8	$41.63
WAR2	1680	144.3	11.6	$23.29
WAR3	5000	333.3	15.0	$30.00
LR1	7000	302.9	23.1	$46.22
LR2	3000	326.9	9.2	$18.35

It is difficult to precisely compare the production value of forest for honey production with the value of the same forest for wood production given the very different nature of the two industries, their respective products and the way those products are marketed and sold. However, the most realistic comparison of leatherwood honey production with wood production is between the farm gate price for honey and the Government royalties paid for saw logs and pulp (J. Hickey Forestry Tasmania, personal communication). For a stand of mixed forest including scattered eucalypts over a mature rainforest sub-canopy, the wood production and royalties from initial clear-felling are approximately 100

m^3 of saw logs (at \$20 per m^3) and 250 m^3 of pulp (at \$15 per m^3) per hectare (J. Hickey, Forestry Tasmania, personal communication). An additional return derives from road-tolls levied by the Government at \$5 per m^3. This gives a total royalty return of \$6250 per hectare of clear-felled forest.

If the clear-felled area is then converted to hardwood plantation, further returns accrue at approximately 300 m^3 of pulp per rotation (at \$25 per m^3 or \$7500 per rotation), plus road-toll returns (at \$5 per m^3 or \$1500 per rotation) giving a total return of \$9000.00 per rotation. Given a single rotation period of approximately 16 years for hardwood plantations, up to 16 rotations could potentially be extracted from one area of land over a period of 250 years (i.e. the flowering life-span of *E. lucida* trees, as above). For 16 full rotations, this would give a gross plantation return of \$144 000 per hectare of forest over 250 years.

The sustainability of hardwood plantation over the long-term has so far not been tested beyond the second rotation (M. Neyland, Forestry Tasmania, personal communication). However, some form of diminishing return through soil exhaustion leading to additional expense to maintain growth rates (e.g. addition of fertiliser) are inevitable. Diminishing returns can be approximated by reducing production value by 10% per rotation. This gives a plantation production value over 250 years assuming a 10% decline in returns per rotation of *ca.* \$73 000, giving a gross wood production return (i.e. initial clear-felling plus 16 hardwood rotations at 10% diminishing return per rotation) of *ca.* \$80 000.

Clearly, according to the above simplistic comparison, the per hectare returns from wood production are vastly superior to honey production. This is the case both for a one-off profit from clear-felling (*ca.* \$6000 per hectare) compared to a single-year return from honey production (*ca.* \$35 per hectare), as well as over the long term (i.e. 250 years) for hardwood plantation (\$80 000 per hectare for 16 rotations at 10 % diminishing returns) compared to 250 seasons of leatherwood honey extraction (*ca.* \$9000 per hectare).

However, a realistic comparison of forest-use practices must consider other factors in addition to simply dollar returns for honey or wood. Commercial apiarists maintain their hives by renting out pollination services to commercial fruit growers during the late winter and spring months. The value of this pollination service to the state is estimated at *ca* 150 million per annum. Furthermore, honey production retains the forest in its native state with a maximal biodiversity value, with the potential for a wide range of recreational usages, and with its inherent aesthetic and spiritual values intact. A realistic and sustainable comparison of forest usage can therefore only be achieved with the inclusion of these other, non-monetary values of Tasmania's native forest.

General Discussion

The commercial production of leatherwood honey in Tasmania involves the placement of 50-150 hives with up to 50 000 bees per hive into substantitively pristine rainforest sites. A single apiary thus involves the injection of upwards of 75 million honeybees into an area of native vegetation. Orthodox niche theory predicts that resources in a stable climax community such as Tasmania's cool temperate rainforests should be fully partitioned (i.e. exploited) among native species which have evolved within the environment (e.g. Pyke 1990). The introduction of very large numbers of honeybees which are both adapted and bred for the rapid harvesting of floral resources would therefore be expected to produce: (a) a substantial increase in the numbers of honeybees working flowers; (b) a decline in the availability of floral resources; and (c) substantial and detectable perturbations in the abundance and activity of native pollinators, and potentially in the reproductive performance (fruit and seed set) of *E. lucida*.

The results of the present study fulfilled only the first and second of these predictions. There was a significant though moderate increase in the number of honeybees foraging at *E. lucida* flowers, and a substantial and significant decline in the availability of both pollen and nectar sugar in the vicinity of apiaries. The relatively modest increase in the number of honeybees observed at flowers even in the vicinity of 100 hives is presumed to be due to the large area over which the bees distribute their foraging effort (around 600-700 hectares; see Chapter 10), and to the presence of significant numbers of feral honeybees at control sites. Despite increasing by a factor of only 2.5 on average, this relatively slight increase in the foraging pressure from honeybees near apiaries nevertheless resulted in a substantial depression in pollen and nectar levels. Clearly, even a small increase in the number of honeybees can exert a powerful effect on the availability of floral resources in an area.

Despite this decline in floral resources around apiaries, there was no overall impact on the abundance or visitation rate of native insects at *E. lucida* flowers. I attribute the absence of any impact on the native fauna to the apparently anomalous over-production of nectar by *E. lucida* trees at a number of the study sites. This superfluity of nectar was very pronounced at several of the sites, with so much nectar produced at one site (QT3) that the nectar was observed dripping from flowers. The reasons for this apparently wasteful over-production of nectar by *E. lucida* flowers are not known, but may relate to the very large variation in the absolute abundance of native insects between ostensibly similar

rainforest sites, and to wide oscillations in weather conditions (and pollinator activity) during anthesis.

If the observed variation in the native insect fauna between sites is stochastic and cannot be 'predicted' by individual trees or flowers, individual *E. lucida* trees may be constrained from an evolutionary standpoint to produce sufficient nectar for the 'best case' scenario of abundant insects. This in turn would lead to a wasteful over-production if a seedling established in a site with few insects. However, because the wind-dispersal distance of *E. lucida* seeds is relatively small (40-150 m; Neyland and Hickey 1990), one might expect strong selective pressure for the adjustment over evolutionary time of nectar production rates to suit local conditions of insect abundance. The fact that many *E. lucida* trees clearly produced a very substantial oversupply of nectar suggests in turn that the time frame for individual *E. lucida* generations (300-400 years) is relatively long compared to the time frame for variation in insect abundance within a site.

Flowers of *E. lucida* produce nectar continuously, and rates of production are independent of ambient temperature and humidity. Nectar sugar accumulates in flowers on cold days (when insects are inactive), and over a series of warm days at sites where insects are scarce. This accumulated nectar sugar is not reabsorbed. As a result, flowers do not appear to adjust (i.e. slow or cease) nectar production at sites where the absolute abundance of insects is very low, or over a series of cold days when insects are inactive. In contrast to nectar production, the rate of anther dehicence is strongly dependent on ambient temperature. The resulting patterns of nectar and pollen production and removal in *E. lucida* flowers appear to provide a flexible and finely tuned mechanism that maximises both male and female function in flowers under a wide range of weather and insect-abundance conditions.

This natural flexibility in *E. lucida's* pollination system may also effectively buffer the species against impacts from hive honeybees. Thus while the introduction of hives to a site may result in a significant alteration in the dynamics of pollen flow between flowers and trees (see Chapter 9), *E. lucida* appears to be sufficiently flexible for such a perturbation to result in minimal net change in reproductive performance.

If flexible pollination systems are characteristic of species growing in highly variable environments (see for example Motten *et al.* 1991), this result for *E. lucida* in Tasmania's cool temperate rainforests may have a more general

application regarding the potential impacts of hive honeybees in other, similarly variable habitats. In effect, impacts of hive bees on the native plant species may be less likely in variable environments in which the native species are adapted to large and stochastic variation in pollinator conditions. In contrast, plant species in habitats with little or highly predictable variation in pollinator conditions may be more subject to perturbations caused by the introduction of large numbers of hive bees.

This study set out to investigate the potential for impacts of hive honeybees on *E. lucida* and its native pollinators. In attempting to address this broad aim, I obtained information on aspects of nectar production and consumption in *E. lucida* flowers, the native pollinator fauna of *E. lucida*, and the breeding system of this cool temperate rainforest species. The results of this study illustrate clearly the importance of obtaining such detailed information on the forage plant species and its native pollinators. Such detailed biological knowledge is absolutely imperative in order to interpret data on the potential impacts of honeybees. Only with a detailed knowledge-base on native plant species is it possible to draw meaningful conclusions as to the existence and extent of honeybee impacts on native biota. This in turn is an essential prerequisite for land managers to make biologically meaningful, practical decisions regarding the acceptability of commercial apiculture in conservation reserves.

Apiculture and impacts in Tasmania's Wilderness World Heritage Area

The primary purpose of conservation areas is the preservation of natural systems. As a result, the licencing of commercial apiculture in reserves poses a potential conflict of interests and philosophies. On the one hand, apiarists view their industry as environmentally friendly and sustainable, and consider nature reserves as a necessary and vital source of economically valuable native forage. As a rule, they consider their industry to have low or negligible impacts on native species (e.g. Manning 1997). On the other hand, the so-called 'green' perspective puts the view that honeybees are an exotic species and therefore an undesirable presence in conservation reserves, and that the introduction of additional hive honeybees during commercial honey production is a contradiction of the basic tenet under-pinning a reserve for the preservation of intact and undisturbed natural systems (New 1997). Caught in the middle are land managers who must make rational and coherent decisions on the availability and extent of reserved land that is made available for commercial apiculture.

The Tasmanian Wilderness World Heritage Area (TWWHA) is 1.38 million hectares in area, covers approximately 20% of Tasmania including the majority of Tasmania's south-west, and includes Tasmania's four largest national parks. Approximately 37% of the total *E. lucida* resource occurs within the TWWHA. Of the state's 144 leatherwood apiary sites, 63 or 14.2% are located within or within 5 km of the TWWHA, with the majority of the remainder located on Crown and private land in the north-west and south of the state. At present, <10% of the total *E. lucida* resource within the TWWHA is accessible for commercial use (Ziegler 1993, and below).

Commercial beekeeping at many of the current leatherwood sites in Tasmania's west and south-west preceded the establishment of both the National Parks and TWWHA. To a large extent, beekeeping has been allowed to continue at these sites, and the current TWWHA Management Plan (1999) permits beekeeping at currently registered sites, but prohibits further expansion into new areas under the 'Precautionary Principle' (Pyke 1990). Further research into honeybee impacts is actively encouraged by the 1999 Management Plan, in the hope that scientifically based data will clarify issues regarding honeybee impacts and the desirability of opening new areas for exploitation.

The results of the present study on the impacts of hive honeybees which are relevant to apiculture management within the TWWHA can be summarised as follows:

- Commercial hives increased the number of honeybees utilising *E. lucida* flowers.
- Hive bees at leatherwood sites foraged almost exclusively on *E. lucida* nectar.
- Hive bees collected pollen from *E. lucida* as well as several other native species, including *Eucalytpus* spp., *Leptospermum* spp. and *A. biglandulosum*.
- Hive honeybees foraged approximately 1.5-2.0 km from the apiary site.
- Within this zone, hive honeybees depressed the amount of nectar sugar and pollen available in *E. lucida* flowers.
- Overall, hive honeybees did not impact on the abundance of native insects or the rate at which native insects visited *E. lucida* flowers.
- However, at four sites where native insects were abundant and the levels of available nectar sugar were low, hive bees may have been reducing the numbers of native insects visiting *E. lucida* flowers.
- Fruit set tended to be higher near apiaries, although other aspects of *E. lucida* reproduction were unaffected by the presence of hive bees.
- However, hive bees may have altered the flow of self and cross pollen between *E. lucida* flowers and trees.

Recommendations for apiculture in TWWHA

The management of commercial apiculture in TWWHA must attempt to balance the primary objectives of the TWWHA and its constituent National Parks (i.e. the conservation of natural systems and values), and the economic and social value of the commercial production of leatherwood honey. Paton (1996) has provided a balanced and objective line of approach to developing a management strategy for honey production in reserves.

Paton (1996) suggests that reserves which have no previous history of regular use by beekeepers should not be opened up for commercial exploitation. For reserves which have a past and ongoing usage for commercial honey production (such as the TWWHA), the balance between honey production and conservation values should be a function of: (a) the extent of the resource which is being utilised within the reserve, and (b) any evidence for impacts of hive honeybees **for that particular reserve.**

Paton (1996; p. 47) recommends a figure of 30% as the minimum area of a resource which should be free of exposure to hive bees, although he notes that this figure is intended to be illustrative and could be higher or lower. For example, Paton's (1996) suggested figure may be more applicable to mainland reserves which tend to be smaller, more accessible and more heavily utilised than is the case for the TWWHA.

If less than the specified minimum percent of a resource is free of exposure to hive bees and/or there are known and clearly delineated impacts of hive bees on some aspect of the natural system within the reserve, then: (a) there should be a mutually agreed program for reducing usage to the specified level within a negotiated time period, and, (b) a mutually agreed program for modifying the existing production process (including number or location of apiary sites, number of hives, spacing of apiaries, and timing of use, or a combination of the above) to reduce the impacts to an acceptable level.

The percentage of the total *E. lucida* resource in the TWWHA which is free of exposure to hive bees was estimated by Ziegler (1993) as approximately 90%. Ziegler (1993) used a foraging range of 3 km radius from the apiary site to calculate the total foraging area of hive bees within the TWWHA. However, hive bees in the present study were found to forage within 2 km of their apiary site (Chapter 10). If a conservative foraging radius of 2.0 km is used, the estimated area of *E. lucida* within the TWWHA which is free of exposure to hive bees is around 95%.

Recommendation 1.

I suggest that a figure of 10% of the total *E. lucida* resource represents a reasonable level of utilisation for the TWWHA reserve system, and that management of apiculture in the TWWHA should aim to maintain a utilisation level which does not exceed 10%. There may therefore be the potential for some expansion of leatherwood honey production within the TWWHA if the new sites are located and considered unlikely to suffer significant impacts (see Recommendation 3).

Because all accessible *E. lucida* is currently fully utilised by commercial apiarists within the TWWHA (Ziegler 1993), any increase in the utilisation of the *E. lucida* resource within the TWWHA would require some novel means of access to additional sites. The construction of new roads is extremely unlikely within the TWWHA. Access could be gained to presently un-utilised *E. lucida*

via helicopter or barge, and applications have in the past been sought for this type of operation. However, approval of these two forms of transport are unlikely to be approved on other grounds (e.g. visual and noise disturbance to wilderness values) than any potential impacts of hive bees *per se*.

An expansion in the level of honey production within the TWWHA could also conceivably be achieved by a more intensive utilisation of the currently accessible resource. The existing distribution of apiary sites within the TWWHA and elsewhere is dictated by a combination of access, existing cleared sites for placing hives, and minimum distances between adjacent sites to avoid competition for the resource. The latter is a point of some contention among apiarists as the precise area covered by an apiary's bees is an unknown quantity and may depend heavily on local topography (see Chapter 10). However, the current minimum distance stipulated by Tasmania's Primary Industries and National Parks regulatory body (i.e. 3 km between apiary sites) is in good agreement with a 1.5-2.0 flight distance recorded in the present study.

Nevertheless, there are occasionally cases of disagreement between commercial operators, with resistance from an established apiarist to the establishment of a new adjacent site by an incoming operator even though the later site is outside the 3 km zone. Furthermore, the results of the present study (see Chapter 8) strongly suggest that sites as little as 2.0 km from even very large apiaries can have very substantial amounts of unutilised nectar available in flowers. This suggests that, at least in some areas, there may be the potential for additional hives to be placed adjacent to existing sites and within 3 km without detriment to the production of the latter.

Recommendation 2.
Where there is the potential for additional hives to be placed in a currently accessible area of the TWWHA, establishment of additional sites be favourably considered provided that the new site/s are considered unlikely to experience significant impacts (see Recommendation 3).

The results from the present study suggest that, while there was no overall detectable impact of hive bees on native insects, there may be a reduction in the number of native insects visiting flowers at sites which have abundant native insects and low levels of available nectar sugar in flowers (see Chapter 8). The latter type of site may be particularly vulnerable to the introduction of commercial loads of hive bees.

Recommendation 3.
Where a new apiary site is proposed within the TWWHA the likelihood that that site will be subject to significant impacts of commercial hives should be assessed. The availability of nectar in *E. lucida* flowers in the late afternoon (1600 hrs) after 2-3 days of warm weather provides a convenient index of the abundance of native insects and the extent of resource utilisation at a site. The availability of nectar can be quickly and accurately assessed using methods described in Appendix 1 and Chapter 8.

Where the level of nectar sugar is <1.0 mg per flower after a 2-3 day period of warm weather, this should be taken as an indication that native insects are relatively abundant at the site and the nectar resource is being fully utilised. Such sites should be kept free of commercial hives. Where the level of nectar sugar is >1.0 mg per flower after a 2-3 day period of warm weather, this should be taken as an indication that native insects are relatively scarce at the site and the nectar resource is not being fully utilised. Such sites should be made available for commercial honey production, provided that Recommendations 1 and 2 are also met.

References

Aizen M. A. & Feinsinger P. (1994a) Habitat fragmentation, native insect pollinators, and feral honeybees in Argentine 'chaco serrano'. *Ecol. App.* **4**, 379-385.

Aizen M. A. & Feinsinger P. (1994b) Forest fragmentation, pollination, and plant reproduction in Chaco dry forest, Argentina. *Ecology* **75**, 330-351.

Armstrong D. P. & Paton D. C. (1990) Methods for measuring amounts of energy available from banksia inflorescences. *Aust. J.Ecol.* **15**, 291-7.

Ashman T. & Stanton M. (1991) Seasonal variation in pollination dynamics of sexually dimorphic *Sidalcea organa* ssp. *spicata* (Malvaceae). *Ecology* **72**, 993-1003.

Ashman T. & Schoen D. L. (1994) How long should flowers live? *Nature* **371**, 788-790.

Augspurger C. (1980) Mass-flowering of a tropical shrub (*Hybanthus prunifolius*): influence on pollinator attraction and movement. *Evolution* **34**, 475-488.

Baker J. D. & Cruden, R. W. 1991. Thrips-mediated self-pollination of two facultative xenogamous wetland species. *Am. J. Bot.* **78**, 959-963.

Barrett S. C. H. & Harder L. D. (1996) Ecology and evolution of plant mating. *Trends Ecol. Evol.* **11**, 73-79.

Bateman A. J. (1948) Intra-sexual selection in Drosophila. *Heredity* **2**, 349-368.

Bawa K. S. (1980) Evolution of dioecy in flowering plants. *Ann. Rev. Ecol. Syst.* **11**, 15-39.

Beardsell D. V., Knox R. B. & Williams E. G. (1993) Breeding system and reproductive success of *Thryptomene calycina* (Myrtaceae). *Aust. J. Bot.* **41**, 333-353.

Becerra J. X. & Lloyd D. G. (1992) Competition-dependent abscission of self-pollinated flowers of *Phormium tenaz* (Agavaceae): a second action of self-incompatibility at the whole flower level? *Evolution* **46**, 458-469.

Bolton A. B., Feinsinger P., Baker H. G. & Baker I. (1979) On the calculation of sugar concentration in flower nectar. *Oecol.* **41**, 301-4.

Bond H. W. & Brown W. L. (1979) The exploitation of floral nectar in *Eucalyptus incrassata* by honeyeaters and honeybees. *Oecol.* **44**, 105-111.

Burd M. (1994) Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Bot. Rev.* **60**, 83-139.

Burquez A. & Corbet S. A. (1991) Do flowers reabsorb nectar? *Funct. Ecol.* **5**, 369-379.

Carothers J. H. & Jaksic F. M. (1984) Time as a niche difference: the role of interference competition. *Oikos* **42**, 403-406.

Carpenter F. L. (1976) Plant-pollinator interactions in Hawaii: pollination energetics of *Metrosideros collina* (Myrtaceae). *Ecology* **57**, 1125-1144.

Charlesworth D. & Charlesworth B. (1987) Inbreeding depression and its evolutionary consequences. *Ann. Rev.Ecol. Syst.* **18**, 237-268.

Collins B. G. & Newland C. (1986). Honeyeater population changes in relation to food availability in the Jarrah forest of Western Australia. *Aust. J. Ecol.* **11**, 63-76.

Collins, B. G. & Spice J. (1986) Honeyeaters and the pollination biology of *Banksia prionotes* (Proteaceae). *Aust. J. Bot.* **34**, 175-185.

Collins B. G., Newland C. & Briffa P. (1984) Nectar utilization and pollination by Australian honeyeaters and insects visiting *Calothamnus quadrifidus* (Myrtaceae). *Aust. J. Ecol.* **9**, 353-365.

Connel J. H. (1980) Diversity and the coevolution of competitors, or the ghost of competition past. *Oikos* **35**, 131-138.

Corbet S. A. (1978) Bee visits and the nectar of *Echium vulgare* L. and *Sinapis alba* L. *Ecol. Ent.* **3**, 25-37.

Corbet S. A., Unwin D. M. & Prys-Jones O. E. (1979) Humidity, nectar and insect visits to flowers, with special reference to *Crataegus, Tilia* and *Echium*. *Ecol. Ent.* **4**, 9-22.

Corbet S. A. & Delfosse E. S. (1984) Honeybees and the nectar of *Echium plantagineum* L in south-eastern Australia. *Aust. J. Ecol.* **9**, 125-139.

Corbet S. A. & Willmer P. G. (1981) The nectar of *Justicia* and *Columnea*: composition and concentration in a humid tropical environment. *Oecol.* **51**, 412-418.

Cresswell W. (1998) Variation in the strength of interference competition with resource density in blackbirds, *Turdus merula*. *Oikos* **81**, 152-160.

Cruden R. W. & Lyon D. L. (1989) Facultative xenogamy: examination of a mixed mating system. In: *The Evolutionary Ecology of Plants* (Eds J. H. Bock & Y. B. Linhart) pp. 171-207. Westview Press, Boulder, Colorado.

Cutis W. M. & Morris D. I. (1981) *The students flora of Tasmania.* Part 1. Second Edition. Government Printer, Hobart, Tasmania.

Darwin C. (1876) *The effects of cross and self-fertilisation in the vegetable kingdom*. Murray, London.

Devlin B. & Stephenson A. G. (1984) Factors that influence the duration of the staminate and pistillate phases of *Lobelia cardinalis* flowers. *Bot. Gaz.* **145**, 323-328.

Donovan B. J. (1980) Interactions between native and introduced bees in New Zealand. *N. Z. J. Ecol.* **3**, 104-116.

Eckert C. G. & Schaefer A. (1998) Does self-pollination provide reproductive assurance in *Aquilegia canadensis* (Ranunculaceae)? *Am. J. Bot.* **85**, 919-924.

Ettershank G. & Ettershank J. A. (1992) *Tasmanian leatherwoods (*Eucryphia *spp.): floral phenology and the insects associated with flowers*. Tasmanian National Rainforest Conservation Program Report No. 11, Forestry Commission, Hobart, Tasmania.

Ettershank G. (1993) Bees and native insects associated with leatherwoods (*Eucryphia* spp.) in Tasmania. *Vic. Nat.* **110**, 251-254.

Faegri K. & van der Pijl L. (1971) *The principles of pollination ecology*. Second Edition. Pergamon Press, Oxford.

Fishbein M. & Venable D. L. (1996) Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. *Ecology* **77**, 1061-1073.

Ford, H. A. (1979) Interspecific competition in Australian honeyeaters – depletion of common resources. *Aust. J. Ecol.* **4**, 145-164.

Frankie G. W, Opler, P. A & Bawa K. S. (1979) Foraging behaviour of solitary bees: implications for outcrossing of a neotropical forest tree species. *J. Ecol.* **64**, 1049-1057.

Freitas B. M & Paxton R. J. (1998) A comparison of two pollinators: the introduced honey bee *Apis mellifera* and an indigenous bee *Centris tarsata* on cashew *Anacardium occidentale* in its native range of NE Brazil. *J. App. Ecol.* **35**, 109-121.

Gibbs D. M. H & Muirhead I. F. (1998) The economic value and environmental impact of the Australian beekeeping inductry. Australian Beekeeping Industry report (un-published)

Gross C. L. & Mackay D. (1998) Honeybees reduce fitness in the pioneer shrub *Melastoma affine* (Melastomataceae). *Biol. Con.* **86**, 169-178.

Harder L. D (1990) Pollen removal by bumble bees and its implications for pollen dispersal. *Ecology* **71**, 1110-1125.

Harder L. D. & Thomson J. D. (1989) Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *Am. Nat.* **133**, 323-344.

Harder L. D. & Barrett C. H. (1992) The energy cost of bee pollination for *Pontederia cordata* (Pontederiaceae). *Funct. Ecol.* **6**, 226-233.

Harder L. D. & Barrett, S. C. H. (1995) Mating costs of large floral displays in hermaphrodite plants. *Nature* **373**, 512-515.

Harder L. D. & Wilson W. G. (1994) Floral evolution and male reproductive success: optimal dispensing schedules for pollen dispersal by animal-pollinated plants. *Evol. Ecol.* **8**, 542-559.

Haynes J. & Mesler M. (1984) Pollen foraging by bumblebees: foraging patterns and efficiency on *Lupinus polyphyllus*. *Oecol.* **61**, 249-253.

Heinrich B. (1976) Resource partitioning among some eusocial insects: bumblees. *Ecology* **57**, 874-889.

Herrera C. M. (1987) Components of pollinator "quality": comparative analysis of a diverse insect assemblage. *Oikos* **50**, 79-90.

Hessing M. B. (1988) Geitonogamous pollination and its consequences in *Geranium caespitosum*. *Am. J. Bot.* **75**, 11324-1333.

Hill R. S. (1991) Leaves of *Eucryphia* (Eucryphiaceae) from Tertiary sediments in south-eastern Australia. *Aust. Syst. Bot.* **4**, 481-97.

Hingston A. (1997). The impact of the large earth bumblebee, *Bombus terrestris* (L.) (Apidae: Apoidea) on Tasmanian ecosystems. Honours thesis, University of Tasmania, Tasmania.

Hingston A. B. & Potts B. M. (1998) Floral visitors of *Eucalyptus globulus* subsp. *globulus* in eastern Tasmania. *Tasforests* **10**, 125-137.

Hodges S. A. (1995) The influence of nectar production on hawkmoth behavior, self pollination, and seed production in *Mirabilis multiflora* (Nyctaginaceae). *Am. J. Bot.* **82**, 197-204.

Holsinger K. E. (1991) Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. *Am. Nat.* **138**, 606-622.

Holsinger K. E. (1996) Pollination biology and the evolution of mating systems in flowering plants. *Evol. Biol.* **29**, 107-149.

Holsinger K. E. & Thomson J. D. (1994) Pollen discounting in *Erythronium grandiflorum*: mass-action estimates from pollen transfer dynamics. *Am. Nat.* **144**, 799-812.

Holsinger K. E., Feldman M. W. & Christiansen F. B. (1984) The evolution of self-fertilization in plants: a population genetic model. *Am. Nat.* **124**, 446-453.

House S. M. (1989) Pollen movement to flowering canopis of pistillate individuals of three rain forest tree species in tropical Australia. *Aust. J. Ecol.* **14**, 77-94.

Human C. & Gordon D. M. (1996) Exploitation and interference competition between the invasive Argentine ant, *Linepithema humile*, and native ant species. *Oecol.* **105**, 405-412.

Hutchinson G. E. (1959). Homage to Santa Rosalia or why are there so many kinds of animals? *Am. Nat* **93**, 145-159.

Inouye D. W & Pyke G. H. (1988) Pollination biology in the Snowy Mountains of Australia: comparisons with montane Colorado, USA. *Aust. J. Ecol.* **13**, 191-210.

Inouye D. W., Gill D. E., Dudash M. R. & Fenster C. B. (1994) A model and lexicon for pollen fate. *Am. J. Bot.* **81**, 1517-1530.

Ishii H. S. & Sakai S. (2000) Optimal timing of corolla abscission: experimental study on *Erythronium japonicum* (Liliaceae). *Funct. Ecol.* **14**, 122-128.

Jackson W. D. (1999) The Tasmanian Environment. In *Vegetation of Tasmania* (Eds J. B. Reid, R. S. Hill, M. J. Brown & M. J. Hovenden.) pp. 11-34. Monotone Art Printers, Hobart, Tasmania.

Jarman S. J. & Brown M. J. (1983) A definition of cool temperate rainforest in Tasmania. *Search* **14**, 81-87.

Jarman S. J., Kantvilas G. & Brown M. J. (1999) Floristic composition of cool temperate rainforest. In *Vegetation of Tasmania* (Eds J. B. Reid, R. S. Hill, M. J. Brown & M. J. Hovenden.) pp. 145-159. Monotone Art Printers, Hobart, Tasmania.

Johnson L. K & Hubbell S. P. (1975) Contrasting foraging strategies and coexistence of two bee species on a single resource. *Ecology* **56**, 1398-1406.

Johnson S. D & Steiner K. E. (2000) Generalization versus specialization in plant pollination systems. *Trends Ecol. Evol.*, **15**, 140-143.

de Jong T. J. & Klinkhamer P. G. L.(1994) Plant size and reproductive success through female and male function. *J. Ecol.* **82**, 399-402.

de Jong T. J., Klinkhamer P. G. L. & van Staaldsuinen M. J. (1992) The consequences of pollination biology for the selection of mass or extended blooming. *Funct. Ecol.* **6**, 606-615.

de Jong T. J., Waser N. M. & Klinkhamer P. G. L. (1993) Geitonogamy: the neglected side of selfing. *Trends Ecol. Evol.* **8**, 321-325.

Juenger T. & Bergelson, J. (2000) Does early season browsing influence the effect of self-pollination in Scarlet Gilia? *Ecology* **81**, 41-48.

Kadmon R. (1992) Dynamics of forager arrivals and nectar rewards in flowers of *Anchusa strigosa*. *Oecol.* **92**, 552-555.

Kalisz S., Vogler D., Fails B., Finer M., Shepard E., Herman T. & Gonzales R. (1999) The mechanism of delayed selfing in *Collinsia verna* (Scrophulariaceae). *Am. J. Bot.* **86**, 1239-1247.

Kearns C. A. & Inouye D. W. (1993) *Techniques for pollination biologists.* University Press of Colorado, Niwot, Colorado.

Kevan P. G. & Baker H. G. (1983) Insects as flower visitors and pollinators. *Ann. Rev. Ent.* **28**, 407-453.

Klinkhamer P. G. L. and de Jong T. J. (1990) Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). *Oikos* **57**, 399-405.

Klinkhamer P. G. L. & de Jong T. J. (1993) Attractiveness to pollinators: a plants dilemma. *Oikos* **66**, 180-184.

Klinkhamer P. G. L., de Jong T. J. & de Bruyn G-J. (1989) Plant size and pollinator visitation in *Cynoglossum officinale*. *Oikos* **54**, 201-204.

Klinkhamer P. G. L., de Jong T. J. & Metz J. A. J. (1994) Why plants can be too attractive – a discussion of measures to estimate male fitness. *J. Ecol.* **82**, 191-194.

Lande R. & Schemske D. W. (1985) The evolution of self-fertilization and inbreeding depression. I. Genetic models. *Evolution* **39**, 24-40.

Lloyd D. G. (1979) Some reproductive factors affecting the selection of self-fertilization in plants. *Am. Nat.* **113**, 67-79.

Lloyd D. G. (1980) Sexual selection in plants. I. An hypothesis of serial adjustment of maternal investment during one reproductive session. *New Phytol.* **86**, 69-79.

Lloyd D. G. (1984) Gender allocations in outcrossing cosexual plants. In: *Perspectives on plant ecology* (Eds R. Dirzo & J. Sarukhan) pp. 277-300. Sinauer, Sunderland, Mass.

Lloyd D. G. (1992) Self and cross-fertilization in plants. II. The selection of self-fertilization. *Int. J. Plant Sci.* **153**, 370-380.

Lloyd D. G. & Schoen D. J. (1992) Self and cross-fertilization in plants. I. Functional dimensions. *Int. J. Plant Sci.* **153**, 358-369.

Lloyd D. G. & Webb C. J. (1986) The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I. Dichogamy. *N. Z. J. Bot.* **24**, 135-162.

Lloyd D. G. & Yates J. M. A. (1982) Intrasexual selection and the segregation of pollen and stigmas in hermaphrodite plants, exemplified by *Wahlenbergia albomarginata* (Campanulaceae). *Evolution* **35**, 903-913.

McFarland D. C. (1985) Flowering biology and phenology of *Banksia integrifolia and B. spinulosa* (Proteaceae) in New England National Park, N.S.W. *Aust. J. Bot.* **33**, 705-714.

Lord E. M. (1981) Cleistogamy: a tool for the study of floral morphogenesis, function and evolution. *Bot. Rev.* **47**, 421-49.

Manning R. (1997) The honey bee debate: a critique of scientific studies of honey bees *Apis mellifera* and their alleged impact on Australian wildlife. *Vict. Nat.* **114**, 13-22.

McFarland D. C. (1985) Flowering biology and phenology of *Banksia integrifolia and B. spinulosa* (Proteaceae) in New England National Park, N.S.W. *Aust. J. Bot.* **33**, 705-14.

McFarland, D. C. (1986) Seasonal changes in the abundance and body condition of honeyeaters (Meliphagidae) in response to inflorescence and nectar availability in the New England National Park, New South Wales. *Aust. J. Ecol.* **11**, 331-40.

McKenna M. A. & Thomson J. D. (1988) A technique for sampling and measuring small amounts of floral nectar. *Ecology* **69**, 1306-7.

Mitchell R. J. & Waser N. M. (1992) Adaptive significance of *Ipompsis aggregata* nectar production: pollination success of single flowers. *Ecology* **73**, 633-638.

Morse D. H. (1974) Niche breadth as a function of social dominance. *Am. Nat.* **108**, 818-830.

Morse D. H. (1977) Resource partitioning in bumble bees: the role of behavioral factors. *Science* 197, 678-680.

Morse D. H. (1981) Interactions among syrphid flies and bumblebees on flowers. *Ecology* **62**, 81-88.

Motten A. F. (1983) Reproduction of *Erythronium umbilicatum* (Liliaceae): pollination success and pollinator effectiveness. *Oecol.* **59**, 351-359.

Motten A. F., Campbell, D. R. & Alexander, D. E. (1981) Pollination effectiveness of specialist and generalist visitors to a North Carolina population of *Claytonia virginica*. *Ecology* **62**, 1278-1287.

Murphy C. & Robertson A. (2000). Preliminary study of the effects of honey bees (*Apis mellifera*) in Tongariro national park. *Science for Conservation* **139**. 18 pp.

New T. R. (1997) Significance of honey bees in the Australian environment: setting the scene. *Vic. Nat.* **114**, 4-7.

Neyland M. & Hickey J. (1990) Leatherwood silviculture - implications for apiculture. *Tasforests* **2**, 63-72.

Nicolson S. W. (1993) Low nectar concentrations in a dry atmosphere: a study of *Grevillea robusta* (Proteaceae) and *Callistemon viminalis* (Myrtaceae). *S. A. J. Sci.* **89**, 473-477.

Nicolson S. W. (1994) *Eucalyptus* nectar: production, availability, composition and osmotic consequences for the larvae of the eucalypt nectar fly, *Drosophila flavohirta*. *S. A. J. Sci.* **90**, 75-79.

Nicolson S. W. (1995) Direct demonstration of nectar reabsorption in the flowers of *Grevillea robusta* (Proteaceae). *Funct. Ecol.* **9**, 584-588.

Nunez J. (1977) Nectar flow by melliferous flora and gathering flow by *Apis mellifera ligustica*. *J. Insect Phys.* **23**, 265-275.

Oldroyd B. P, Lawler S. H. & Crozier R. H. (1994) Do feral honey bees (*Apis mellifera*) and regent parrots (*Polytelis anthopeplus*) compete for nest sites. *Aust. J. Ecol.* **19**, 444-450.

Ollerton J. (1996) Reconciling ecological processes with phylogenetic patterns: the apparent paradox of plant-pollinator systems. *J. Ecol.* **84**, 767-769.

Osorio-Beristain M., Dominguez C. A, Eguiarte L. E. & Benrey B. (1997) Pollination efficiency of native and invading Africanized bees in the tropical dry forest annual plant, *Kallstroemia grandiflora* Torr ex Gray. *Apidologie* **28**, 11-16.

Parker C. H. (1995) Submission to consultative committee: beekeeping in World Heritage Area. Un-published report.

Paton D. C. (1985) Food supply, population structure, and behaviour on New Holland Honeyeaters *Phylidonyris novaehollandiae* in woodland near Horsham. In *Birds of Eucalypt forests and woodlands: ecology, conservation, management* (Eds A. Keast, H. F. Recher, H. Ford & D. Saunders) pp. 219-230. Surrey Beatty & Sons, Sydney.

Paton D. C. (1986) Honeyeaters and their plants in south-eastern Australia. In *The dynamic partnership: birds and plants in southern Australia* (Eds H. A. Ford & D. C. Paton) pp. 9-19. South Australian Government Printer, Adelaide.

Paton D. C. (1993) Honeybees and the Australian Environment. *Bioscience*. **43**, 95-103.

Paton D. C. (1996) Overview of the impacts of feral and managed honeybees in Australia: distribution, abundance, extent of interactions with native biota, evidence of impacts and future research. Invasive Species Program, Australian Nature Conservation Agency, Canberra.

Paton D. C. (1997). Honey bees *Apis mellifera* and the disruption of plant-pollinator systems in Australia. *Vict. Nat.* **114**, 23-29.

Paton D. C. (1999). Impacts of commercial honeybees on flora and fauna in Ngarkat Conservation Park. RIRDC Publication No. 99/15, ACT.

Paton D. C. & Turner V. (1985) Pollination of *Banksia ericifolia* Smith: birds, mammals and insects as pollen vectors. *Aust. J. Bot.* **33**, 271-286.

Pflumm W. (1985) Influence of nectar-supply rate on the number of flowers visited by a honeybee on each collecting flight. *Oecol.* **66**, 207-210.

Pleasants J. M. & Chaplin S. J. (1983) Nectar production rates of *Asclepias quadrifolia*: causes and consequences of individual variation. *Oecol.* **59**, 232-238.

Plowright R. C. (1981) Nectar production in the boreal forest lily *Clintonia borealis*. *Can. J. Bot.* **59**, 156-160.

Price M. V. & Waser N. M. (1982) Experimental studies of pollen carryover: hummingbirds and *Ipomopsis aggregata*. *Oecol.* **54**, 353-358.

Pyke G. H. (1990) Apiarists versus scientists: a bittersweet case. *Aust. Nat. Hist.* **23**, 386-92.

Pyke G. H. (1985) The relationship between abundances of honeyeaters and their food resources in open forest near Sydney. In: *Birds of Eucalypt forests and woodlands: ecology, conservation, management* (Eds A. Keast, H. F. Recher, H. Ford & D. Saunders) pp. 65-77. Surrey Beatty & Sons, Sydney.

Pyke G. H. & Balzer L. (1985) The effects of the introduced honeybee (*Apis mellifera*) on Australian native bees. Occasional Papers No. 7, NSW National Parks and Wildlife Service, Sydney.

Pyke G. H. & Recher, H. F. (1986) Relationship between nectar production and seasonal patterns of density and nesting of resident honeyeaters in heathland near Sydney. *Aust. J. Ecol.* **11**, 195-200.

Rademaker M. C. J., de Jong T. J. & van der Meijden E. (1999) Selfing rates in natural populations of *Echium vulgare*: a combined empirical and model approach. *Funct. Ecol.* **13**, 828-837.

Ramsey M. W. (1988) Differences in pollinator effectiveness of birds and insects visiting *Banksia menziesii* (Proteaceae). *Oecol.* **76**, 119-124.

Ramsey M. (1995) Ovule pre-emption and pollen limitation in a self-fertile perennial herb (*Blandfordia grandiflora*, Liliaceae). *Oecol.* **103**, 101-108.

Ramsey M. & Vaughton, G. (2000) Pollen quality limits seed set in *Burchardia umbellata* (Colchicaceae). *Am. J. Bot.* **87**, 845-852.

Ramsey M., M., Prakash N. & Cairns S. (1993) Breeding system of disjunct populations of Christmas Bells (*Blandfordia grandiflora* R.Br., Liliaceae): variation in self-fertility and an ovular mechanism regulating self-fertilisation. *Aust. J. Bot.* **41**, 35-47.

Reid J. (1989) Phenology and germination in some rainforest canopy species at Mt Field National Park, Tasmania. *Pap. Proc. Roy. Soc. Tas.* **123**, 211-221.

Read, J. 1999. Rainforest ecology. In: *Vegetation of Tasmania* (Eds J. B. Reid, R. S. Hill, M. J. Brown & M. J. Hovenden.) pp. 160-197. Monotone Art Printers, Hobart, Tasmania.

Richardson T. E. & Stephenson A. G. (1989) Pollen removal and pollen deposition affect the duration of the staminate and pistillate phases in *Campanula rapunculoides*. *Am. J. Bot.* **76**: 532-538.

Robertson A. W. (1992) The relationship between floral display size, pollen carryover and geitonogamy in *Myosotis colensoi* (Kirk) Macbride (Boraginaceae). *Biol. J. Linn. Soc.* **46**, 333-349.

Roubik, D. W. (1978) Competitive interactions between neotropical pollinators and africanized honey bees. *Science* **201**, 1030-1032

Roubik, D. W. (1980) Foraging behavior of competing Africanized honeybees and stingless bees. *Ecology* **61**, 836-845.

Roubik, D. W. (1983) Experimental community studies: time-series tests of competition between African and neotropical bees. *Ecology* **64**, 971-978.

Roubik, D. W., Moreno J. E, Vergara C. & Wittmann D. (1986) Sporadic food competition with the African honey bee: projected impacts on neotropical social bees. *J. Trop. Ecol.* **2**, 97-111.

Schaffer W. M., Jensen D. B., Hobbs D. E., Gurevitch J., Todd J. R. & Schaffer, M. V. (1979) Competition, foraging energetics, and the cost of sociality in three species of bees. *Ecology* **60**, 976-987.

Schaffer W. M., Zeh D. W., Buchmann S. L., Kleinhans S., Schaffer M. V. & Antrim J. (1983) Competition for nectar between introduced honey bees and native North American bees and ants. *Ecology* **64**, 564-577.

Schemske D. W & Horvitz C. C. (1984) Variation among floral visitors in pollination ability: a precondition for mutualism specialization. *Science* **225**, 519-521.

Schoen D. L. & Lloyd D. G. (1984) The selection of cleistogamy and heteromorphic diaspores. *Biol. J. Linn. Soc.* **23**, 303-322.

Schoen D. L. & Brown A. H. D. (1991) Whole- and part-flower self-pollination in *Glycine clandestina* and *G. argyrea* and the evolution of autogamy. *Evolution* **45**, 1651-1664.

Schoener, T. W. (1974) Resource partitioning in ecological communities. *Science* **185**, 27-38.

Schoener T. W. (1983) Field experiments on interspecific competition. *Am. Nat.* **122**, 240-285.

Schwarz M. P. & Hurst P. S. (1997) Effects of introduced honey bees on Australia's native bee fauna. *Vict. Nat.* **114**, 7-12.

Schwarz M. P., Gross C. L. & Kukuk P. F. (1992) Assessment of competition between honeybees and native bees. WWF Australia Progress Report.

Seavey S.R. & Bawa K. S. (1986) Late-acting self-incompatibility in Angiosperms. *Bot. Rev.* **52**, 195-219.

Silander J. A. & Primack R. B. (1978) Pollination intensity and seed set in the Evening Primrose (*Oenothera fruticosa*). *Am. Mid. Nat.* **100**, 213-216.

Southwick E. E. (1984) Photosynthate allocation to floral nectar: a neglected energy investment. *Ecology* **65**, 1775-1779.

Spears E. E. (1983) A direct measure of pollinator effectiveness. *Oecol.* **57**, 196-199.

Sugden E. A. & Pyke G. (1991) Effects of honey bees on colonies of *Exoneura asimillima*, an Australian native bee. *Aust. J. Ecol.* **16**, 171-181.

Tasmanian Wilderness World Heritage Area Plan (1999). Parks and Wildlife Service, Department of Primary Industries, Water and Environment. Hobart, tasmania.

Steinbachs J. E. & Holsinger K. E. (1999) Pollen transfer dynamics and the evolution of gametophytic self-incompatibility. *J. Evol. Biol.* **12**, 770-78.

Stephenson A. G. (1981) Flower and fruit abortion: proximate causes and ultimate functions. *Ann. Rev. Ecol. Syst.* **12**, 253-279.

Sutherland S. (1986) Patterns of fruit-set: what controls fruit-flower ratios in plants? *Evolution* **40**, 117-128.

Sutherland S. & Delph L. F. (1984) On the importance of male function in plants: patterns of fruit-set. *Ecology* **65**, 1093-1104.

Thomson J. D. (1986) Pollen transport and deposition by bumble bees in *Erythronium*: influences of floral nectar and bee grooming. *J. Ecol.* **74**, 329-341.

Thomson J. D. and Plowright R. C. (1980) Pollen carryover, nectar rewards, and pollinator behavior with special reference to *Diervilla lonicera*. *Oecol.* **46**, 68-74.

Thomson J. D., McKenna M. A,. & Cruzen M. B. (1989) Temporal patterns of nectar and pollen production in *Aralia hispida*: implications for reproductive success. *Ecology* **70**, 1061-1068.

Vaughton G. (1988) Pollination and seed set of *Banksia spinulosa*: evidence for autogamy. *Aust. J. Bot.* **36**, 633-642.

Vaughton G. (1992) Effectiveness of nectarivorous birds and honeybees as pollinators of *Banksia spinulosa* (Proteaceae). *Aust. J. Ecol.* **17**, 43-50.

Vaughton G. & Ramsey M. (1991) Floral biology and inefficient pollen removal in *Banksia spinulosa* var. *neoanglica* (Proteaceae). *Aust. J. Bot.* **39**, 167-177.

Waser N. M. & Price M. V. (1991) Reproductive costs of self-pollination in *Ipomopsis aggregata* (Polemoniaceae): are ovules usurped? *Am. J. Bot.* **78**, 1036-1043.

Waser N. M., Chittka L, Price M. V., Williams N. M. & Ollerton J. (1996) Generalization in pollination systems, and why it matters. *Ecology* **77**, 1043-1060.

Webb C. J. & Lloyd D. G. (1986) The avoidance of interference between the presentation of pollen and stigmas in angiosperms. II. Herkogamy. *N. Z. J. Bot.* **24**, 163-178.

Wenner A. M. (1992) Estimating effective foraging ranges of honey bee colonies. *Am. Bee J.* **12**, 817-818.

Whelan R. J. & Burbidge A. H. (1980) Flowering phenology, seed set and bird pollination of five Western Australian *Banksia* species. *Aust. J. Ecol.* **5**, 1-7.

Wiens D., Calvin C. L., Wilson C. A., Davern C. I., Frank D. & Seavey S. R. (1987) Reproductive success, spontaneous embryo abortion, and genetic load in plants. *Oecol.* **71**, 501-509.

Williams G. A. & Adam P. (1997) The composition of the bee (Apoidea: Hymenoptera) fauna visiting flowering trees in New South Wales lowland subtropical rainforest remnants. *Proc. Linn. Soc. N.S.W.* **118**, 69-95.

Williams G. A. & Adam P. (1998) Pollen loads collected from large insects in Australian subtropical rainforests. *Proc. Linn. Soc. N. S. W.* **120**, 49-67.

Wilson M. F. (1979) Sexual selection in plants. *Am. Nat.* **113**, 777-790.

Wilson P. & Thomson J. D. (1991) Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. *Ecology* **72**, 1503-1507.

Wyatt R., Broyles S. B. & Derda G. S. (1992) Environmental influences on nectar production in milkweeds (*Asclepias syriaca* and *A. exaltata*). *Am. J. Bot.* **79**, 636-642.

Young H. J. & Stanton M. L. (1990) Influences of floral variation on pollen removal and seed production in wild radish. *Ecology* **71**, 536-547.

Ziegler K. I. (1993) Leatherwood nectar resource management report. Forests and Forest Industry Council, Tasmania.

Zimmerman M. & Pyke G. H. (1988) Pollination ecology of Christmas bells (*Blandfordia nobilis*): patterns of standing crop of nectar. *Aust. J. Ecol.***13**, 301-9.

Ziv Y., Abramsky Z., Kotler B. P. & Subach A. (1993) Interference competition and temporal and habitat partitioning in two gerbil species. *Oikos* **66**, 237-246.

Appendix 1. A technique for washing nectar from flowers of *E. lucida*

Techniques for extracting and measuring floral nectar are an important requirement for most studies of plant-pollinator interactions (Kearns and Inouye 1993). The standard equipment are fixed-bore micropipettes for extracting nectar, and hand-held refractometers for measuring percentage sugar (Bolton *et al.* 1979). Although the micropipette/refractometer technique is rapid and simple, it is poorly suited to flowers where nectar is produced in very small volumes and/or where the nectar is highly viscous. A number of alternative techniques have been developed to cope with such situations. These include using filter-paper wicks to blot up small quantities of dilute or viscous nectar (McKenna and Thomson 1988), homogenisation of flowers (Nunez 1977), centrifuging inflorescences (Armstrong and Paton 1990), and the washing of nectar from flowers (Nunez 1977; Collins *et al.* 1984; Zimmerman and Pyke 1988).

The nectar of *E. lucida* is utilised by commercial apiarists in the production of leatherwood honey. Ettershank and Ettershank (1992; also Ettershank 1993) attempted to quantify the volume and concentration of nectar produced by *E. lucida* flowers. They used a standard micropipette/refractometer method to extract and measure nectar sugar, where 5μL micropipettes were inserted around the bases of the anthers and the nectar withdrawn through capillary action. Using this technique, small to moderate quantities of relatively dilute nectar were obtained before 1000 hours (5.7-15.4μL at 15.6-18.1% wt/wt), with each flower estimated to contain approximately 1.5mg of sugar. However, after mid-morning, nectar could not be obtained from either exposed flowers or flowers protected from insects by fine-mesh nylon bags. Ettershank and Ettershank (1992) concluded that nectar was produced at night, resulting in moderate quantities of dilute nectar in the early morning which was then dehydrated to >70% over the heat of the day.

In an attempt to confirm the results of Ettershank and Ettershank (1992), I used 5μL micropipettes to extract liquid nectar from flowers picked just after dawn (0600 hours). Nectar volumes were typically very small (<4μL maximum; mean±se=1.1±0.23μL), with many flowers yielding no nectar. However, flowers yielding no nectar were clearly still attractive to nectarivorous insects, suggesting that the nectar was being rapidly dehydrated, making it too viscous to extract using micropipettes.

I therefore developed an alternative wash-technique to extract this concentrated nectar from flowers. The study was conducted at WAR1 and LR1 in the summer of 1998 (see Study Sites section). Flowers were gathered from

trees on the edges of roads and tracks, which typically flowered to near ground level. Over a period of four weeks in January and February 1998, a total of 87 *E. lucida* flowers were picked during daylight hours and placed anthers-up in a wooden block in which a series of holes (5 mm radius) had been drilled to support the flower base. Using a pair of tweezers, the central gynoecium of each flower was broken off at the base, and 20μL of distilled water from a 20μL micropipette were added to the space made by the removal of the ovary. This added water tended to puddle at the bases of the clustered stamens. The flowers were left standing for 5- 10 minutes, after which the liquid was withdrawn using a 20μL micropipette, with the nectar volume in the flower considered to be the amount of solution extracted over and above the original 20μL (Zimmerman and Pyke 1988). The concentration of the extracted solution was then measured using a hand-held refractometer (Atago models N1: 0-32% and N2: 28-62% wt/wt; Tokyo, Japan; and Universal Type model no. 505-I: 0-90% wt/wt, Iwaki, Japan: all measurements adjusted to 20°C), and a second 20μL quantity of distilled water was added to each flower. This procedure was continued for each flower until the concentration of the extracted liquid either equaled zero, or gave two consecutive readings of < 0.2%. The number of washes needed to remove all nectar sugars from a flower ranged from two to five.

Using Table 5-2 in Kearns and Inouye (1993), I expressed all wt/wt readings as g solute per 100 ml solution (Bolton *et al.* 1979), from which the weight of sugar per wash could be calculated by adjusting for the added 20μL of water. Occasionally during extraction of the wash fluid, I obtained slightly less than the original 20μL, presumably due to small quantities of fluid clinging by surface tension to the tissues of the flower. Where this was the case, I assumed that the solution extracted came from a pool of approximately 20μL, even though only a portion of this total pool was actually removed.
For each flower, the total weight of sugar removed for all washes was calculated, and the amount of sugar removed per wash expressed as a percentage of this total (Fig. App.1.1). The first wash removed approximately 83% of total sugar, the second wash approximately 12% of total sugar, while subsequent washes each removed <5% of total sugar (Fig. App.1.1). Using two washes per flower therefore removed approximately 95% of total sugar, and provided a quick and simple field technique for estimating floral sugar in *E. lucida* flowers with small quantities of viscous nectar. While there was some potential for error in the sugar estimation due to small volumes of wash-fluid remaining in the flower, the volume of fluid lost was generally small (<3μL) relative to the total added (20μL). The technique was independent of the concentration of nectar in flowers, as the wash-fluid dissolved even the highly

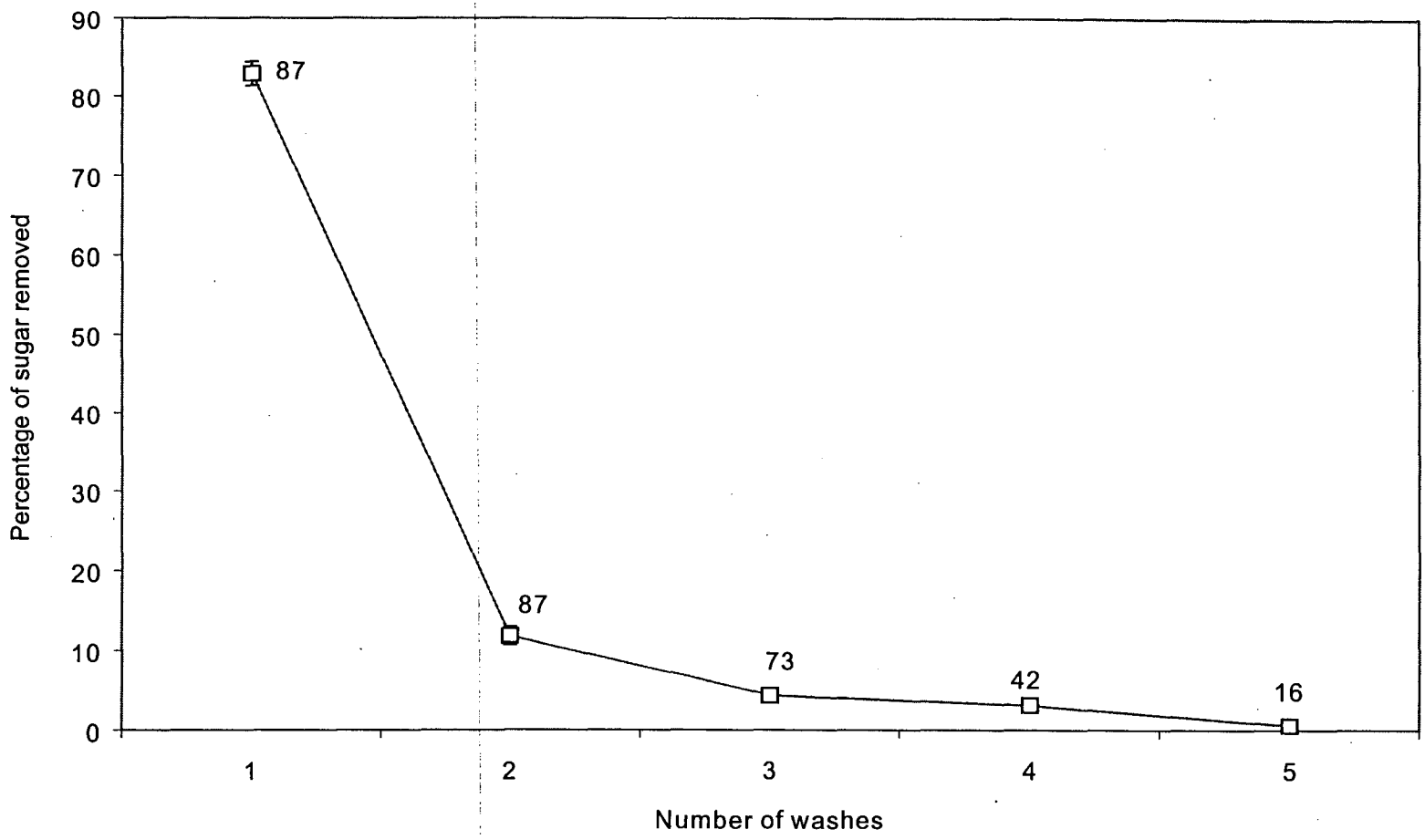

Fig. App.1.1. Percentage of total floral sugar removed from *E. lucida* flowers by repeated washes. Error bars are standard errors. Samples sizes shown.

viscous nectar present in flowers after 1000 hours, allowing an accurate estimate of total sugar weight per flower where nectar could not be extracted using micropipettes.

Appendix 2. Impacts of hive honeybees at sites with abundant native insects and low levels of available nectar sugar in flowers (WAR1, WAR2, WAR4 and WAR5)

Despite a significant reduction in available nectar sugar in flowers around apiaries, I found no evidence for any 'flow-on' effects on the abundance of native insects at *E. lucida* flowers. This was attributed to an apparent over-production of nectar at a number of sites where visitation rates by native insects were extremely low. In Chapter 8, I suggested that hive honeybees might have a demonstrable impact on native nectivorous insects at sites where the background levels of native insects are high and the majority of the daily nectar sugar production is consumed by flower visitors. These conditions were satisfied at four sites studied in a single block in February 1998, two apiary sites (WAR1 and WAR2) and two control sites (WAR3 and WAR4). Because these sites were studied simultaneously (i.e. sample days and times were the same for all sites; see Chapter 8), they also provide a useful means of investigating honeybee impacts while including sample day as a fixed factor in the experimental design.

See Chapter 8 Methods section for details of data collection methods. I used a hierarchical ANOVA to analyse the flower-scan and nectar data. Data were log-transformed to satisfy conditions of normality and equality of variance. It could be argued that days is a random effect, which would alter the denominators in some tests. However, I chose to keep days as a fixed effect as there was evidence of substantial differences among days for some variables. Where there was evidence of differences among days, I grouped days according to similar means and analysed these data separately until the effect of day was no longer significant.

The video data included a large number of zeros, and were not amenable to a hierarchical ANOVA analysis (see Chapter 8). I therefore pooled the video data for different days and used a two-way ANOVA on the mean values for each site to test for an effect of apiary and time of day on mean visitation rates by honeybees, large dipterans, and total native insects. For the sticky-trap data, I used a students t-test to test for an effect of apiary on the mean number of insects per trap.

All data are presented as mean±se.

Results

Honeybees

There was a significant effect of apiary on the number of honeybees per 500 flowers, with significantly more honeybees recorded at flowers at the apiary sites compared to control sites ($F_{1,59}$=24.54, P<0.05 (Fig. App. 2.1). There was also a significant effect of day ($F_{4,59}$=3.91, P<0.05) and time of day ($F_{2,59}$=7.69, P<0.01) on the number of honeybees per 500 flowers. Based on the similarities in their mean values, I grouped days 1-2 and days 3-5. For Days 1-2, there was a significant effect of the presence of an apiary ($F_{1,23}$=149.66, P<0.01) and of time of day ($F_{2,23}$=12.62, P<0.01), but no effect of day ($F_{1,23}$=0.03, P>0.5). For Days 3-5, the effect of the presence of an apiary was close to significant ($F_{1,35}$=9.06, 0.05<P<0.1) as was the effect of time of day ($F_{2,35}$=3.22, 0.05<P<0.1), while there was no effect of day ($F_{2,35}$=0.03, P>0.5). For all days and times of day combined, the mean number of honeybees per scan for two apiary and two control sites were 6.1±1.0 and 1.0±0.3, respectively.

There was a significant effect of apiary on the rate of honeybee visits to flowers ($F_{1,6}$=11.90, P<0.025) but no effect of time of day ($F_{2,6}$=1.56, P>0.2), with a greater rate of honeybee visits to flowers at the apiary sites (Fig. App. 2.2). For all days and times of day combined, mean visitation rates by honeybees to *E. lucida* flowers were 0.17±0.03 visits/flower/10-minutes for the two apiary sites, and 0.07±0.01 visits/flower/10-minutes for the two control sites.

Nectar

Although nectar sugar tended to increase at the two control sites and remain relatively low at the two apiary sites (Fig. App. 2.3), there was no overall effect of apiary ($F_{1,717}$=8.19, P>0.1) or time of day ($F_{2,717}$=0.75, P>0.4) on floral sugar levels. However, the effect of day was highly significant ($F_{4,717}$=17.34, P<0.001), with substantially more nectar present in flowers on Days 1 and 2 compared to Days 3-5. There was no effect of the presence of apiary or time of day on Day 1 ($F_{1,142}$=1.31, P>0.3 and $F_{1,142}$=1.49, P>0.3, respectively) or Day 2 ($F_{1,149}$=0.25, P>0.6 and $F_{1,149}$=2.91, P>0.1, respectively). For Days 3-5, the effect of the presence of an apiary was close to significant ($F_{1,424}$=17.02, 0.05<P>0.1), while there was no effect of time of day ($F_{2,424}$=0.0.9, P>0.4) or day ($F_{1,424}$=1.60, P>0.3).

Native insects at flowers

For large dipterans, the effect of apiary was close to significant ($F_{1,6}$=5.25, 0.05<P>0.1), as was the effect of time of day ($F_{2,6}$=4.94, 0.05<P>0.1), with the

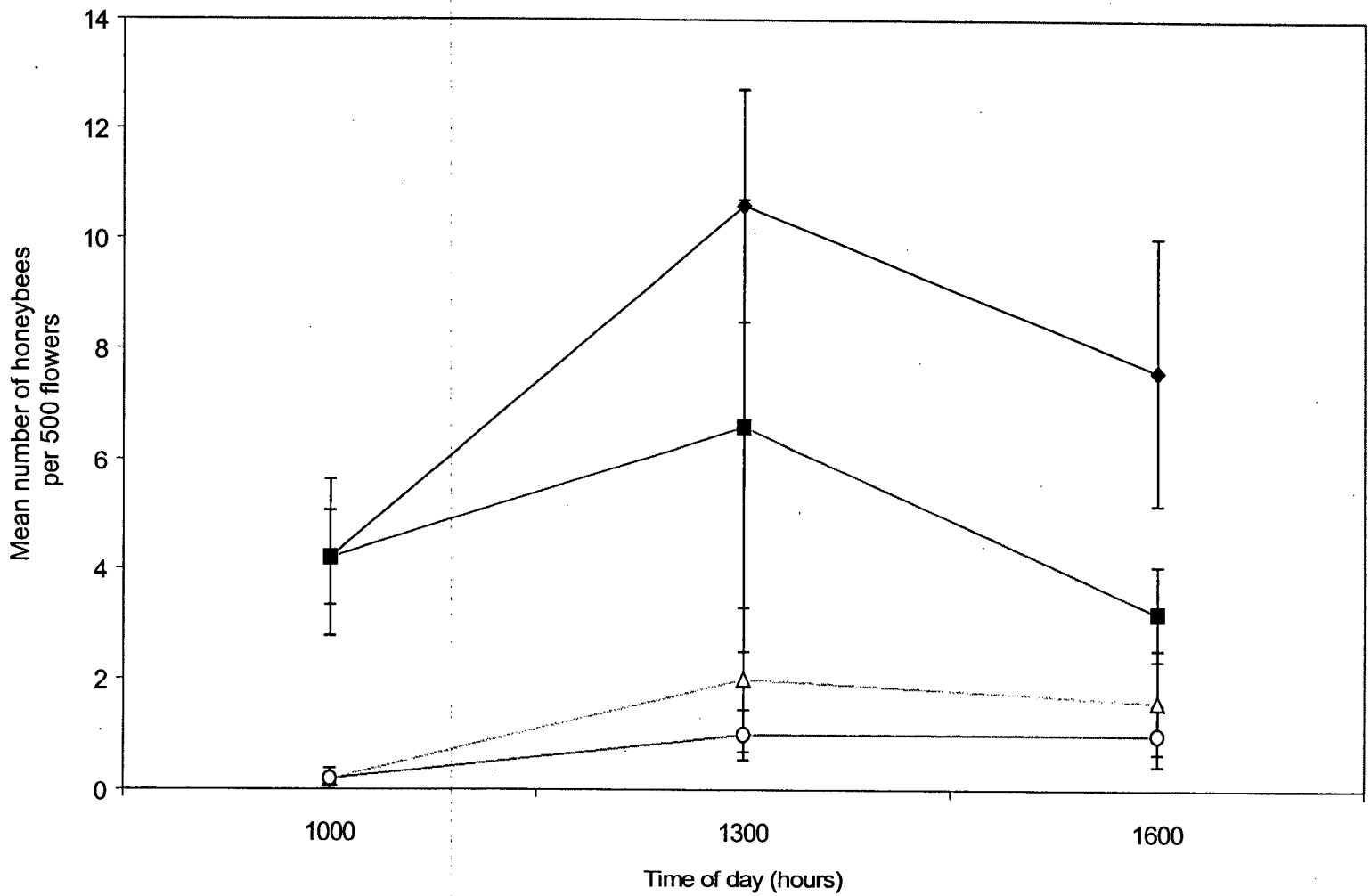

Fig. App. 2.1. Mean number of honeybees recorded during scans of 500 flowers over the day at the four Waratah sites. Filled symbols are apiary sites, open symbols are control sites. WAR1 - squares, WAR2 - diamonds, WAR3 – triangles, WAR4 – circles. n=5 for all points. Error bars are standard errors.

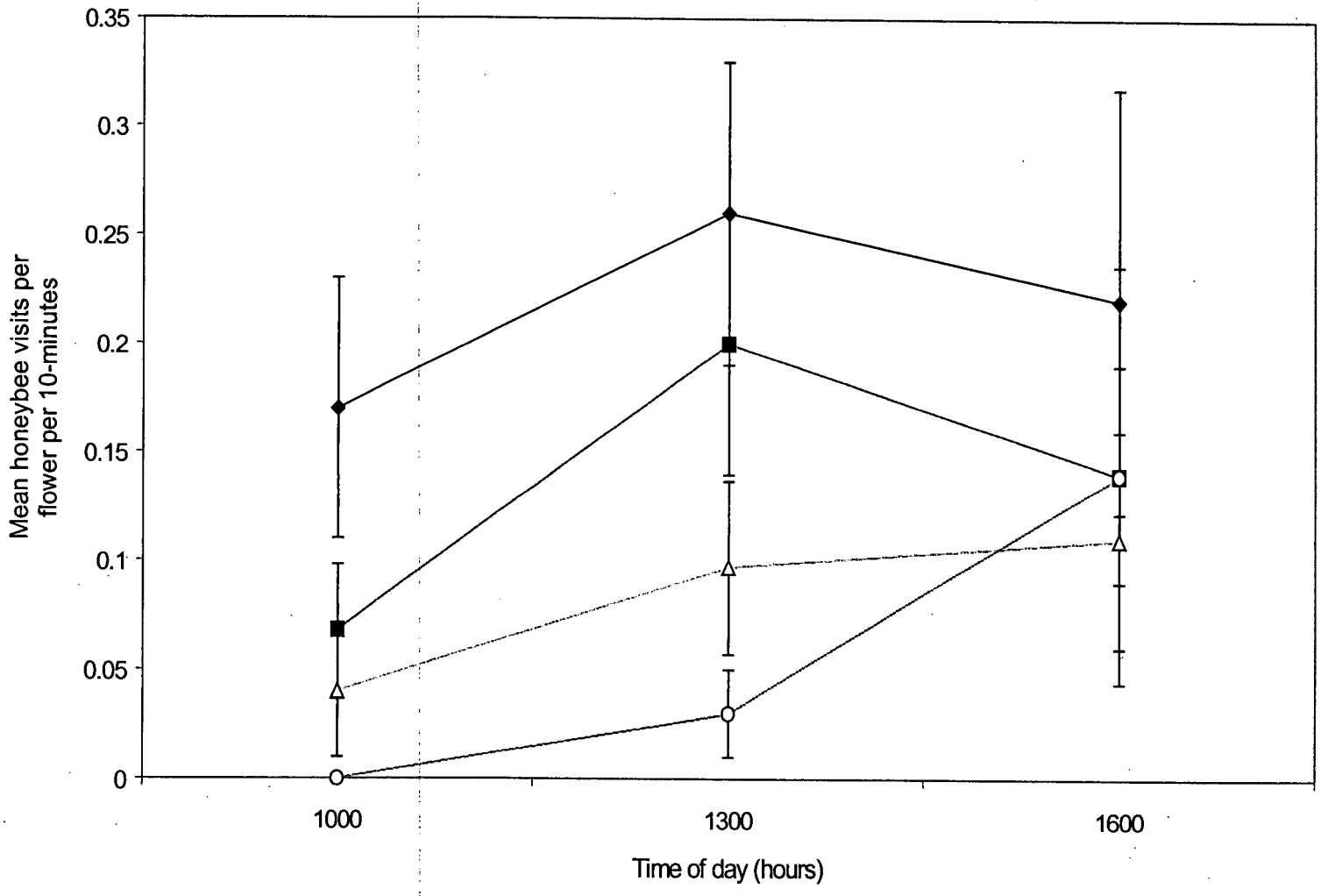

Fig. App. 2.2. Mean number of honeybee visits per *E. lucida* flower per 10-minutes over the day at the four Waratah sites. Filled symbols are apiary sites, open symbols are control sites. WAR1 - squares, WAR2 - diamonds, WAR3 – triangles, WAR4 – circles. n=15-24 for all points. Error bars are standard errors.

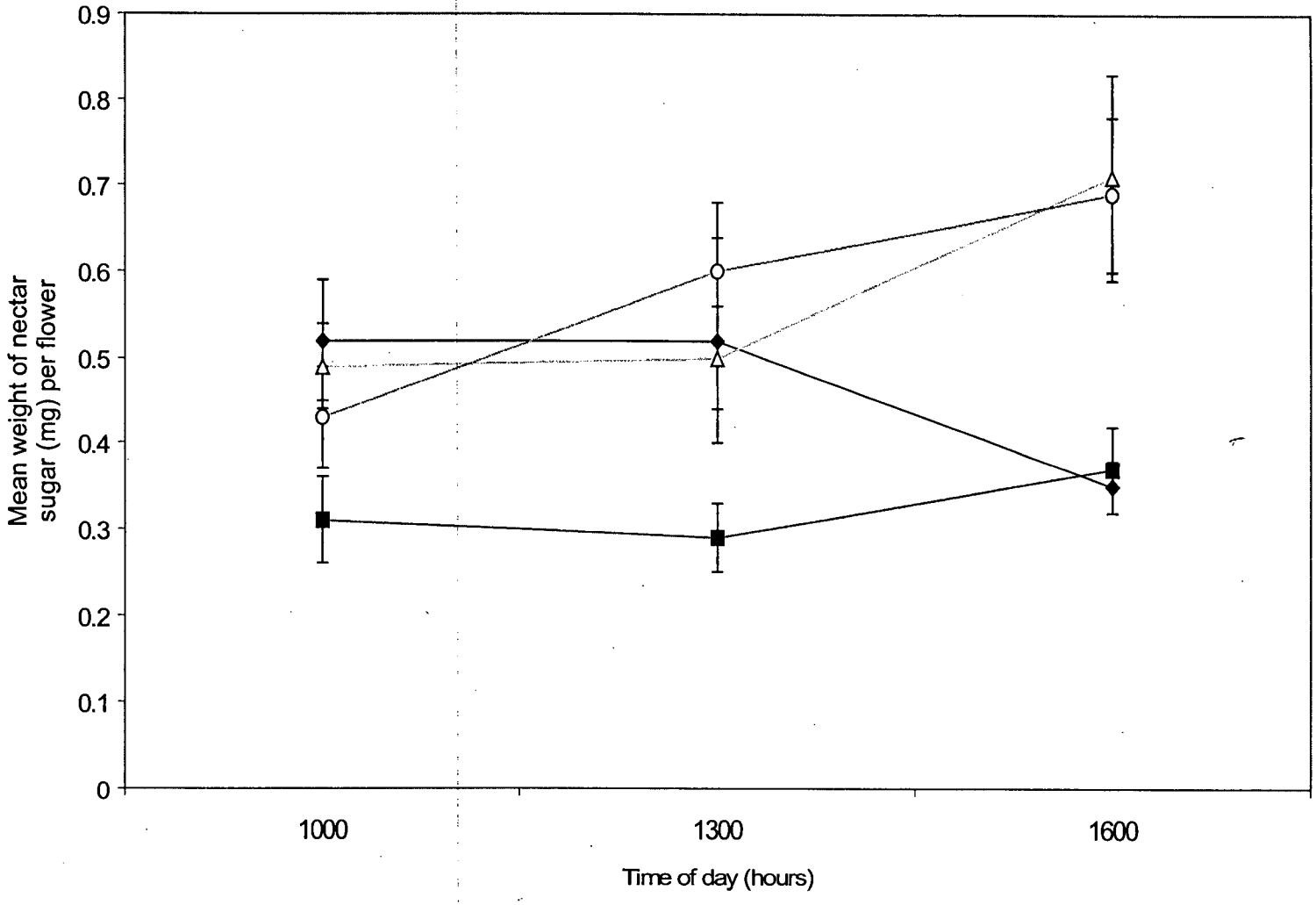

Fig. App. 2.3. Mean weight of nectar sugar per flower over the day at the three Waratah sites. Filled symbols are apiary sites, open symbols are control sites. WAR1 - squares, WAR2 - diamonds, WAR3 – triangles, WAR4 – circles. n=58-66 for all points. Error bars are standard errors.

visitation rate by large dipterans tending to be higher at 1300 hours and 1600 hours at the control sites (Fig. App. 2.4). For total native visitors, there was a significant effect of apiary ($F_{1,6}$=6.42, P<0.05) and time of day ($F_{2,6}$=7.17, P<0.05) and a significant interaction between apiary and time of day ($F_{1,6}$=8.01, P<0.025), with more native insects visiting flowers later in the day at the control sites (Fig. App. 2.5). For the three times of day combined, the visit rates for both large dipterans and total natives were higher at the two control sites (0.23±0.04 and 0.31±0.04 visits/flower/10-minutes, respectively) compared to the two apiary sites (0.14±0.02 and 0.20±0.03 visits/flower/10-minutes, respectively).

Invertebrate sampling

There was no significant differences between apiary and control sites in the number of large dipterans or total native insects caught on sticky-traps (unpaired t-tests, t_2=0.95, P>0.4 and t_2=0.92, P>0.4, respectively) (see Fig. 8.8a,b, Chapter 8).

Discussion

The trend for increased honeybee activity around apiaries observed for all study sites (Chapter 8) was clearly apparent at the four Waratah sites considered here. The number of honeybees recorded per 500 flowers increased by a factor of 6 at apiary sites, while the visitation rate by honeybees increased by a factor of 2.4 at apiary sites (Fig. App. 2,2). Similarly, nectar sugar levels tended to be lower near apiary sites for the four Waratah sites, although the trend was less pronounced than for other site comparisons (see Table 8.1, Chapter 8). The reason for a less pronounced difference in nectar sugar levels presumably reflects the relatively large numbers of native insects visiting flowers at the control sites WAR4 and WAR5 (see Fig. 8.6a,b, Chapter 8), and the relatively low production of nectar sugar at these sites (Table 8.1, Chapter 8). In addition, there was a significant tendency for visitation rates by native insects to be lower at the two apiary sites later in the day when the levels of available nectar were depressed (Fig. App. 2.5). This indicates that, at least for these four study sites, an increased number of hive bees and a resulting depression in available nectar sugar around apiary sites may result in a decline in the number of native insects visiting flowers. However, there was no difference in the numbers of native insects on sticky traps between the two apiary and two control sites, although the statistical power of the test was severely limited by the small number of replicates.

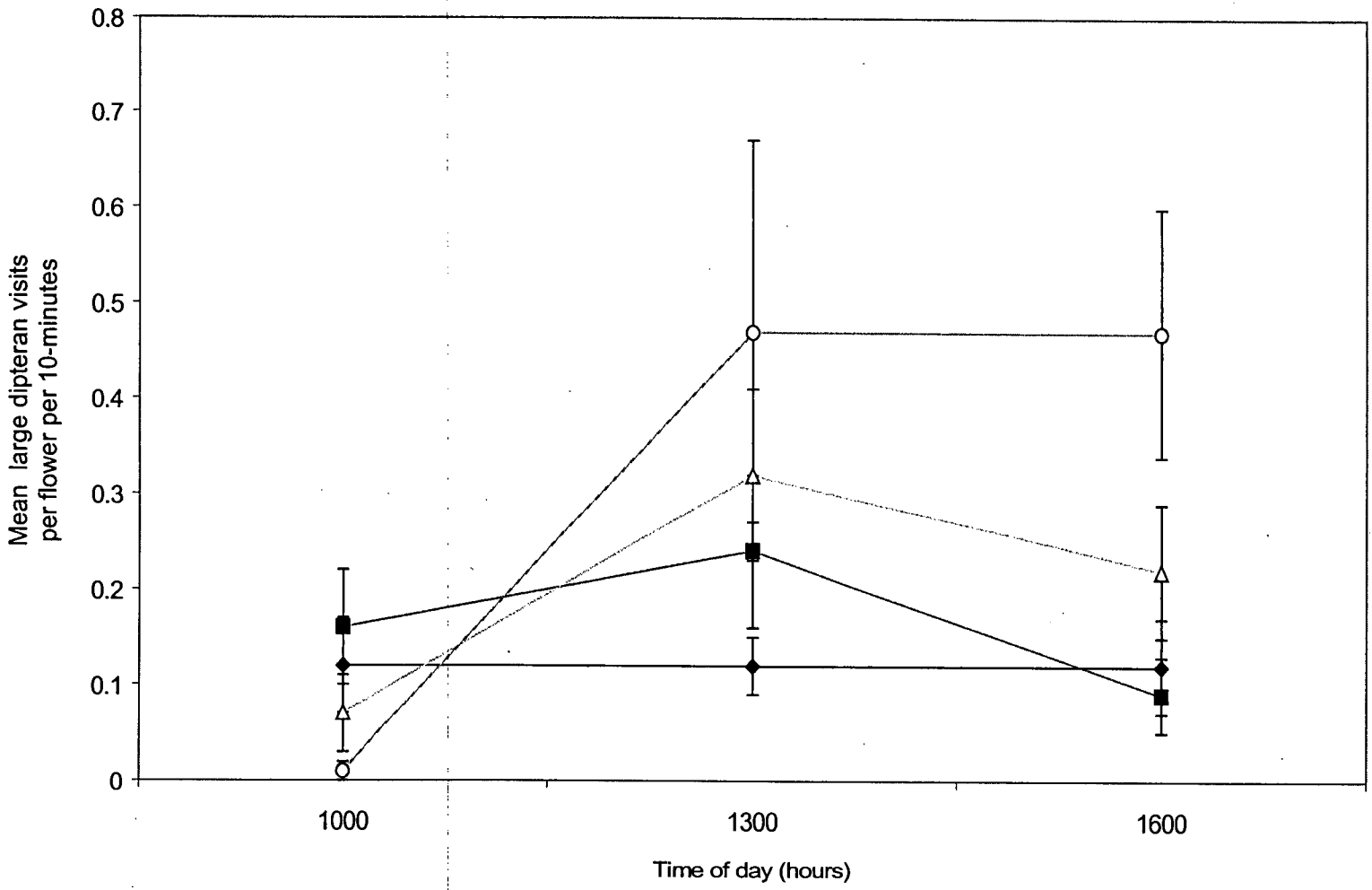

Fig. App. 2.4. Mean number of large dipteran visits per *E. lucida* flower per 10-minutes over the day at the four Waratah sites Filled symbols are apiary sites, open symbols are control sites. WAR1 - squares, WAR2 - diamonds, WAR3 – triangles, WAR4 – circles. n=15-24 for all points. Error bars are standard errors.

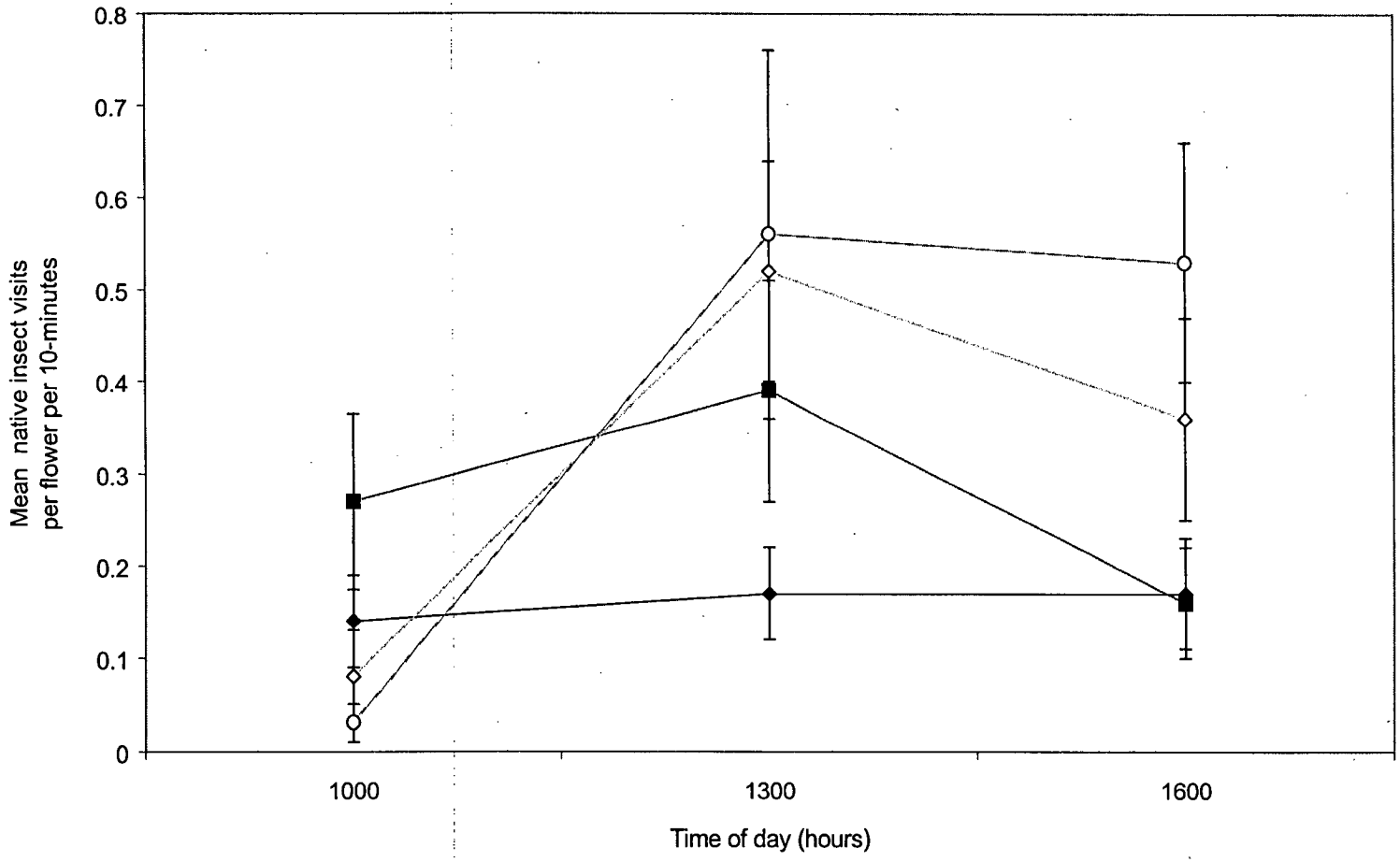

Fig. App. 2.5. Mean number of native insect visits per *E. lucida* flower per 10-minutes over the day at the four Waratah sites Filled symbols are apiary sites, open symbols are control sites. WAR1 - squares, WAR2 - diamonds, WAR3 – triangles, WAR4 – circles. n=15-24 for all points. Error bars are standard errors.

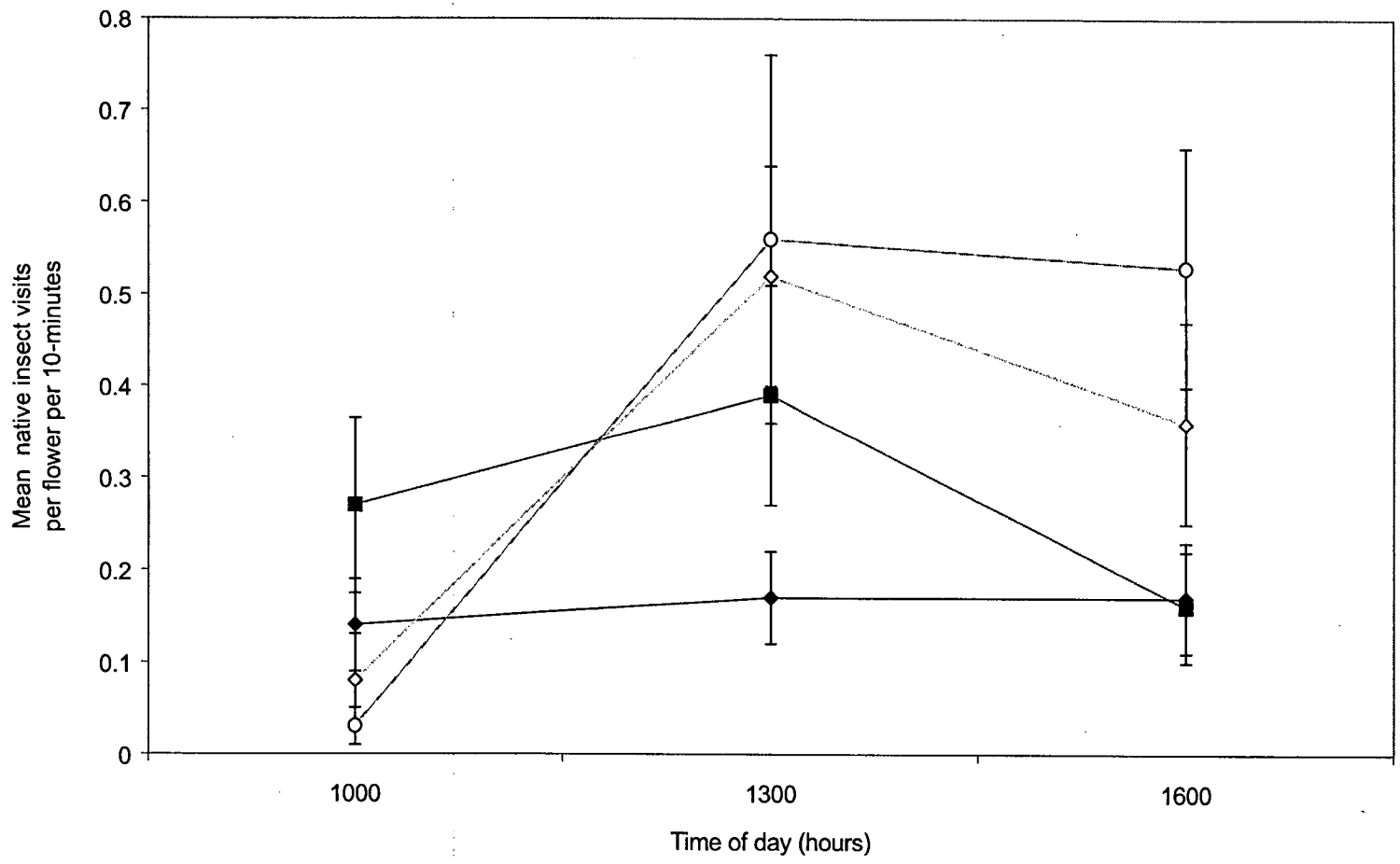

Fig. App. 2.5. Mean number of native insect visits per *E. lucida* flower per 10-minutes over the day at the four Waratah sites Filled symbols are apiary sites, open symbols are control sites. WAR1 - squares, WAR2 - diamonds, WAR3 – triangles, WAR4 – circles. n=15-24 for all points. Error bars are standard errors.

In summary, for all study sites I found that apiaries depressed nectar sugar but had no effect on the number of native insects visiting flowers (see Chapter 8). In contrast, for a specific comparison of the four Waratah sites, there was a strong suggestion that hive bees were depressing nectar levels *and* reducing the number of native insects at flowers. I suggest that such an impact of commercial apiaries on native insects may be characteristic of sites where the background levels of native insects are high and nectar sugar is contained at relatively low levels - that is, at sites without an over-supply of nectar sugar. If so, then the potential for impacts of hive bees on native insects will be a function of the characteristics of the site to which the hives are introduced. At sites with very low background numbers of native insects and a super-abundance of *E. lucida* nectar available in flowers, introducing commercial hives to the forest may have no impact on the native insect fauna. In contrast, sites with abundant native insects and low levels of available nectar sugar in flowers may be subject to significant impacts. Further work on the impacts of apiaries on nectar and native insects at the latter type of site is needed to test this hypothesis.